Symposia
Within the UEMS and closely linked to the European Society of Vascular Surgery there has been a yearly examination in vascular surgery since 1996. The aim has been to have a common control of knowledge among vascular surgeons within Europe. Today vascular surgery has become a section of its own and passing the examination renders the candidate to become a fellow. When the examination started it consisted of three parts: first a case discussion (one case with extremity ischaemia, one with aneurysmal disease and one miscellaneous), secondly discussion of a scientific paper to check the candidate’s ability to understand the message, the scientific quality of the paper and to draw the correct conclusions, thirdly a discussion based on the log book when applying for the examination. In all three parts the questions are structured and the answers defined to avoid any bias as much as possible. From 2003 a fourth part has been included in the examination, that is a practical part consisting of three stations: a groin dissection of the sapheno-femoral junction to perform a high ligation, a test of knot tying and the skill to perform an anastomosis between a synthetic graft and a crural artery. In the years 2006 and 2007 a fifth part was tested with the aim to check the endovascular skill of the candidate. This part will be included in the examination from 2008. Two countries, where vascular surgery recently has become a subspeciality, have decided to use the European examination as their exit examination to obtain the speciality. Others will probably follow. This will mean some logistic problems in the future with the need to sit the examination on two or more occasions yearly, which will need more examiners, which in turn will increase the costs of the examination. In the table is shown the passing rate so far:

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<tr>
<th>Year</th>
<th>City</th>
<th>No of candidates</th>
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<td>13</td>
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<td>1997</td>
<td>Lisbon</td>
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<td>London</td>
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<td>2001</td>
<td>Lucerne</td>
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<td>2006</td>
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<td>Madrid</td>
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<td></td>
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<td>253</td>
<td>206 (81%)</td>
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Pathophysiology of Haemostasis and Thrombosis

JS.2: Vascular Centres: The Role on Training

Fabrizio Benedetti-Valentini

Dpt. of Vascular Surgery, La Sapienza, University, Rome, Italy

“Dedicated centre where patients with vascular disease can receive high quality medical, endovascular and open surgical treatment by appropriate experts working as a coordinated team “ it is the definition given by the Joint Committee (JC) of the Section of Vascular Surgery of the UEMS and of the International Union of Angiology.

The main functions of a VC are a range of qualified angiological, radiological and surgical services, vascular consulting service including vascular laboratory, 24h 365d service for emergencies and cooperation with primary care physicians. The VC should be a complex and complete organization, a high volume caring institution, serving and adequate territory and mass of population, located in a main hospital, University or General Hospital, where all kind of service and professional collaboration is readily available.

Under such conditions a VC is the best Training Centre (TC) for all vascular professions. Setting some final points the JC states that “training and research are essential functions of a vascular centre”. There are many guidelines coming from the main vascular societies and many national or european directives concerning the training of a Vascular Specialist but all of them emphasize a high and articulate exposure of the trainee to diagnosis and treatment of the vascular patients. There is also a current debate whether to train a vascular physician, an endovascular therapist and a vascular surgeon or a vascular specialist with multiple expertise. In some countries of the EU Angiology and Vascular Surgery are part of the same speciality, in some others Angiologists practice currently endovascular treatments, in some others they are trying to join Intervascular Radiology and Vascular Surgery. May be that different ways are best for different countries but what is certain it is that as high volume and multidisciplinary institution the VC will be the best place to train any kind of vascular specialist.
Vascular surgery (VS) was approved as a subspecialty in United States in 1982. Initially, one year of fellowship training was required after complete general surgery (GS) training (5 clinical years). By 2000, most vascular fellowships had expanded to 2 clinical years, to allow sufficient endovascular training in addition to open surgery. In 2005, VS was approved as an independent specialty, such that prerequisite GS certification is not required. This allowed vascular programs to develop 2 new training paradigms, approved in 2006. The first of these is a 5 clinical year program beginning immediately after medical school. Two of these years comprise GS rotations, while 3 comprise VS rotations, including the final chief residency year. Training is under the supervision of the VS program director, and applicants match during their fourth year of medical school. Upon completion, these residents will be eligible for certification in VS only. The second new paradigm consists of 3 years preliminary GS training followed by 3 years VS training, of which only the last 3 years are under the supervision of the VS program director. This paradigm was designed for potential future use, if surgery as a whole transitions to preliminary training followed by specialty training, but at this point it is not being pursued. There are now 10 accredited 5 year vascular training programs, and this is already a very popular and competitive choice for medical student applicants. Each program has devised their own curriculum, to introduce trainees to VS early in their experience, and to eliminate unnecessary GS rotations, while focusing the GS rotations on trauma, critical care, and abdominal surgery. Thus, in United States there are now two major pathways for a VS training, one the traditional two year VS fellowship following GS residency, leading to certification in both GS and VS, and the new 5 year VS residency program leading to certification in VS only. The latter seems attractive to many medical students who have made an early decision about their VS career choice. Providing both options preserves later career choice during residency, while the new 5 year programs provide a more efficient method that may attract new trainees.
Introduction

The purpose is to discuss the training of vascular surgeons in the Scandinavian countries. The training program is under revision in some countries, and in Sweden it is about to be established.

A concern is decreasing recruitment to vascular surgery in several countries. Another problem is that the workload of endovascular therapy is increasing, while the training programs have not been adapted to these realities. Since there is no uniform Scandinavian model for the training in vascular surgery, each country will be mentioned separately.

Sweden

Vascular surgery will most likely be organised as a sub-speciality based on general surgery, which is common in the Scandinavian countries. Thus, three years of training in vascular surgery will become necessary. Some of this time, probably 1 year, can be included in the training for general surgery, provided the new program is adopted by the Health Authorities. There are plans to introduce an operating list also including endovascular procedures. Courses in both general and vascular surgery should be followed. An examination is still not compulsory. The plan is to adopt the European exam (EBSQ Vasc).

Finland

In Finland vascular surgery is based on a common trunk of three years. An additional three years of vascular surgical training is necessary to become a specialist. The candidates must fill in a log book similar to what is used for the UEMS examination. In general the trainees get more operations than needed. An endovascular program is under preparation. Eighty hours of courses in vascular surgery are needed in addition to a 20 hour course in administration. There is an oral examination at the end of the common trunk, and a written examination after the vascular surgical training. There are 23 vascular surgeons with this new training in Finland while 34 are specialists in thoracic and vascular surgery, with a vascular profile.

Iceland

In Iceland, a revision of the training program is planned. At present, 4 1/2 years of training in general surgery and 1/2 year in anaesthesiology are necessary to become a specialist in general surgery. Thereafter two years of work at a department of vascular surgery is
necessary to become a sub-specialist in vascular surgery.

**Denmark**

Denmark was the first Scandinavian country to establish vascular surgery as a speciality (mono speciality). Following 1-1 1/2 years of internship, the specialization is starting with 12 months of basic surgery. The training in vascular surgery includes 60 months as a resident. The candidate should have experience from two separate departments of vascular surgery. Included in these five years there is half a year of common trunk, which probably will be removed. Then there are rotations of 2-4 weeks duration, which include orthopaedic surgery, diabetology, cardiology, interventional radiology etc. Three of the five years should be spent as a senior resident at a department of vascular surgery. In Denmark one has a personal mentor to follow that the requirements to become a vascular surgeon are met. There is an operating list consisting of altogether 340 vascular surgical procedures including diagnostic arteriography (30) and PTA with or without stenting (20). There is a progressive responsibility for the specialist candidate and the more difficult operations are performed during the last part of the training.

**Norway**

In Norway vascular surgery is also a sub-speciality of general surgery. Three years of vascular surgery is needed. Six of years of training in general surgery is needed, but two of these years can be spent in a department of vascular surgery and therefore count as specialisation in vascular surgery. Therefore seven years of training is the minimum to become a specialist. Eighty hours of courses in vascular surgery must have been attended. The candidate has a personal mentor. The mentor has a meeting with the candidate every three month. In addition there are teaching programs two hours weekly in each institution. The operating list includes 240 procedures and endovascular therapy has recently been included. However, 220 of the operations are open procedures. There is no speciality in angiology in Norway and therefore the vascular surgeons have to take care of diagnosis, including duplex ultrasound, and medical therapy of patients with vascular diseases. When on call, the specialist candidates also cover general surgery (vascular, GI, urology, paediatric, trauma, endocrine etc). With the regulation of working hours and the organisation of on call duty, which is forcing the candidates to take a day off after having been on call, there is less focus on vascular surgery than desired. There is no examination in vascular surgery in Norway.

**Conclusion**

There is no uniform “Scandinavian model” for training in vascular surgery. However, usually vascular surgery is a sub-speciality based on general surgery and the vascular surgeon needs to be a specialist both in general and vascular surgery. Courses in vascular surgery are compulsory in most countries. In some of the programs much of the time is spent on general surgery. The volume of endovascular therapy needs to be increased in the training program of most Scandinavian countries. Perhaps 1/2 - 1 years of training in this discipline should be included in near future. We feel that simulators should be introduced in the training, although we still need hard data to prove the benefit of this form of training operative skills. A centralisation of vascular surgery could, in some countries, lead to a higher volume of procedures for the specialist candidates. On the positive side there is an operating list that the candidates need to complete prior to approval, and a personal mentor is checking that the requirements to become a specialist are fulfilled. The basis in general surgery might be advantageous in countries with long distances and scattered population. However, in the future there will probably be more specialisation than today.
Patients with mechanical heart valves, atrial fibrillation or thromboembolic disease have to be under long term anticoagulation therapy for protection from thromboembolism.

A dilemma arises for physicians when OACT (oral anticoagulation therapy) patients need to undergo elective or minor surgery. In these situations the risk of bleeding, if the procedure is performed without interruption of OACT, has to be counterbalanced with thrombosis risk associated with OACT discontinuation.

The options available in this case are:

a) to continue providing OACT
b) to stop providing OACT some time before and after the operation.
c) or, as a third management strategy, maintain a degree of anticoagulation during perioperative period using a strategy called bridging therapy

This refers to the use of parenteral shorter acting anticoagulants at a therapeutic dose namely UFH (unfractionated heparin) or LMWH (low molecular weight heparin) after temporarily withdrawal of OACT.

To avoid bleeding risk, most surgeries can be performed when the INR is below 1.5. Such levels are achieved after nearly 3-5 days of OACT discontinuation and there is a need of 3 or more days to reach INR values in therapeutic range, when warfarin is restarted. Residual anticoagulant effect from warfarin is a major problem in these patients and needs further testing and study.

A potential procedure of bridging is as following: OACT is stopped 3-5 days prior to surgery, administration of heparin starts 2 days prior to the procedure. The initial heparin dose is periodically adjusted to a target level of aPTT 1.5 to 2 times the normal range. Intravenous UFH is usually interrupted 4 hours before surgery and restarted 12 to 24 hours according to the bleeding risk.

Subcutaneously LMWH has been proposed as an attractive, effective and safe alternative anticoagulant therapy. In comparison to UFH is more convenient and of lower cost, mainly due to the administration in outpatients.

In case of emergency surgery, we must take into consideration the specific pharmacokinetic properties of VKA (vitamin-K antagonists), the patient’s characteristics (haemorrhagic versus thromboembolic risks) and surgical factors.

It is crucial to differentially evaluated multiple clinical situations that necessitate optimal bridging therapy. It has to be mentioned that so far for all the above, there has not been enough documentation, and there are more questions to be answered, concerning doses, starting providing points, time of treatment and target population.

In conclusion we should be able to properly individualize treatment, balancing between benefits and risks of bridging therapy in patients at risk.
References


Since 1-2% of the western population are on coumarin anticoagulants (mostly warfarin), the request for advice on the management of the bleeding patient is a frequent occurrence for any haematologist. The two main issues influencing the mode of reversal are the INR and the severity of bleeding. Discontinuation of warfarin is rarely useful in isolation because it will take 3 days or more for an adequate fall in the INR. Vitamin K is very useful in reducing the INR. Given intravenously it will reduce the INR within 6-8 hours, whilst with the oral preparation this can be achieved within 24 hours. For major or life-threatening bleeding the only effective method to reverse the anticoagulation is with the use of prothrombin complex concentrate. These products contain factors II, IX, X and variable amounts of FVII and following their administration the INR can be normalised within 5 minutes. Despite the widespread use of FFP in this situation, its efficacy is poor and can not be recommended for this indication.

Patients bleeding on unfractionated heparin, rarely require treatment other than discontinuation of the infusion. Bleeding on full dose low molecular weight heparin can be partially reversed with protamine sulphate. Patients who are still bleeding despite protamine can be treated with NovoSeven.

Fondaparinux is difficult to reverse and protamine has no efficacy in this setting. In vitro and very limited in vivo data suggest that NovoSeven may be useful in this situation.
Pathophysiology of Haemostasis and Thrombosis

**SYM I.3: Management of oral anticoagulant overdose in asymptomatic patients**

George Theodossiades
First Regional Transfusion and Haemophilia Centre, Hippocration Hospital, Athens, Greece

Oral anticoagulant agents—vitamin K antagonists—(VKAs), are indicated for use in cases of atrial fibrillation, deep vein thrombosis and valve replacement. Around 1% of the population will be taking VKAs for one of these indications. Clinical trials indicate that the risk of a major haemorrhage resulting from their usage ranges from 0.5 to 4.2 cases per 100 patients/year. To minimize the risk of VKAs complications, the prothrombin time (PT) – generally expressed as the international normalized ratio (INR) to allow comparability among centers – is used to monitor the degree of VKAs-associated anticoagulation. Despite monitoring and careful dose adjustment, overanticoagulation with or without bleeding is frequently seen. An excessively elevated INR independently predicts major bleeding; the risk of bleeding approximately doubles for each single point of increase in the INR above 3.0 and major bleeding occurs in about 4% of patients who present with an INR of more than 6.0 who are treated with simple warfarin withdrawal.

The clinical management of patients who are receiving long-term oral anticoagulant therapy and who have supratherapeutic INR values is a frequently encountered and problematic clinical scenario. The objectives of treatment for non-bleeding patients with an excessively prolonged INR value is to reduce the likelihood that the patient will develop bleeding complications by reducing the duration of INR prolongation, while minimizing the risk of thrombosis with a sub-therapeutic INR. Two main treatment strategies are available: withholding drug alone, or withholding drug and administering vitamin K. A third option, administering vitamin K and transfusion therapy of coagulation factors (either in the form of FFP or coagulation factor concentrates) should not be routinely used in non-bleeding patients because it is expensive, potentially risky and has not been demonstrated to be more effective than simple administration of vitamin K. In order to decide between these two options, it is important, at first, to take into account the half-life of the oral anticoagulant used (acenocoumarol has the shortest half-life of 10 hours, warfarin is intermediate with 35 to 45 hours, and phenprocoumon has the longest half-life of 3 to 5 days), and then for the selection of an appropriate dose of vitamin K, the route of administration, the current INR level, and the target INR for the next few days. In most cases, the oral administration of 1 to 2 mg of vitamin K is adequate to bring a moderately high INR level of 4.5 to 9 down to the therapeutic range. For higher INR levels, 5 to 10 mg of vitamin K should be given.
Low molecular weight heparins (LMWH) have been developed from unfractionated heparins (UFH) using various chemical procedures. The origin of the starting material has been found to be crucial for the biological activity of the LMWH preparations. The patents for LMWHs are now expiring and biosimilar LMWHs are in development. Biosimilar LMWHs are currently being produced by different companies with the aim to reproduce the original compounds enoxaparin, dalteparin and tinzaparin. No standard criteria for the determination of the similarity between originator and biosimilar LMWH’s are currently available.

The scientific subcommittee (SSC) on anticoagulation of the International Society of Thrombosis and Haemostasis (ISTH) has set up a working group to develop guidelines for preclinical and clinical requirements of biosimilar LMWHs to demonstrate their similarity with the originator product. At present, biosimilar products of enoxaparin are in development and the working group focuses on this specific anticoagulant. Participants of the expert group are Trevor Barrowcliffe, David Bergqvist, Benito Casu, Jawed Fareed, Job Harenberg, Eric Holmer, Ajay Kakkar, Patrick Mismetti, Barbara Mulloy, Wolfram Raake, Michel Samama, and Sam Schulman.

The preliminary results of the discussion of the working group will be presented. Participants of the scientific session will be asked to fill out prepared questions and statements on a form which will be available at the session. The opinion of the participants of the scientific session will be included into the guidelines of the working group of the SSC on anticoagulation of the ISTH.
Pathophysiology of Haemostasis and Thrombosis

SYM II.2: Structural and biochemical profiling of low-molecular weight heparins and their biosimilars

Benito Casu and Giangiacomo Torri

G. Ronzoni Institute for Chemical and Biochemical Research, Milan, Italy

Although the internal structure of LMWH chains is largely unaffected by the most common depolymerization processes, different approaches to cleavage of the parent heparins mainly determine the type of terminal groups, the average size of the fragments, and dispersion of molecular weights around the average values. A first-level characterization of LMWHs accordingly involves identification and quantification of terminal groups, determination of the average MW and MW dispersion. A second, important level, requires determination of the antithrombin-binding sequences (AT-bs). Though contained in only about one fifth of the chains constituting LMWHs, these sequences are major contributors to the anti-Xa activity of LMWHs. Since the AT-bs may be affected by the depolymerization processes, assessment of the percent affinity for AT is considered an important requirement for the characterization of LMWHs and their biosimilars.

Compositional profiling of LMWHs can be obtained either by LC-MS analysis of the disaccharide / oligosaccharide mixtures obtained by exhaustive chemical or enzymatic depolymerization, or directly by NMR spectroscopy. Substantial identity of these profiles are thought to be a prerequisite for assessing the identity of a LMWH and its biosimilars. More complete structural information can be obtained through analysis of two-dimensional (HQSC) NMR spectra, which permits to quantify different sulfation patterns in terms of individual, variously sulfated, monosaccharide and disaccharide residues, including the typical ones in the AT-bs. Ongoing studies are focused on structural analysis of fractions with high affinity and no affinity for AT obtained by affinity chromatography on size-homogeneous tetra- to hexadecasaccharide fractions of the most common LMWHs.
Low molecular weight heparins are prepared from pig intestinal lining.

The quality of raw material may vary as recently illustrated by adverse reactions in patients who could be attributed to a raw ingredient.

Differences in pharmacodynamic and pharmacokinetic characteristics of LMWH are well documented. They may play a role in their efficacy and safety in different populations of patients.

In patients treated with LMWH for deep vein thrombosis (DVT) or pulmonary embolism (PE) it has been shown by us and others that the peak values of anti-Xa activities are clearly different demonstrating the differences in the half-life plasma clearance of anti-Xa activity.

In contrasts, anti-IIa activity is significantly increased when comparing Tinzaparin to other LMWHs. Similar observations have been made regarding TFPI release, the role of renal clearance etc...

Heparin immunogenicity may also vary from one LMWH to another or a biosimilar preparation.
Pathophysiology of Haemostasis and Thrombosis

**SYM II.5: Biostatistic design for studies to compare originator and generic low-molecular-weight heparins on the incidence of immunogenic response or postoperative venous thromboembolism**

Patrick Mismetti

Thrombosis Research Group, University hospital of Saint-Etienne, France

Due to the strong relationship between the manufacturing process and the characteristics of the final products, generic LMWH can not be considered as generic equivalents of innovator LMWH. So the demonstration of the equivalent bioavailability between generics and innovators assessed by classical pharmacodynamic markers (anti-factorXa activity) is not sufficient. In accordance with the EMEA guidelines regarding biosimilars, we need also to demonstrate the comparability of the generic and innovator LMWH in terms of immunogenicity, efficacy and safety.

However, to be less expansive than the innovator, the immunogenicity testing and the benefit to risk assessment have to use surrogate endpoint in a relevant population and indication in order to be able to extrapolate these results on the different indications of the innovator LMWH.

The immunogenicity testing of generic LMWH has to be performed in humans but could be easily compared to the innovator LMWH by using the determination of a platelet factor IV heparin antibody (PF4) and by using a non inferiority hypothesis on this parameter. However the potential immunogenicity of a generic LMWH can not be resumed to PF4 antibodies and we need to define a priori a program of pharmacovigilance during the post-marketing period.

To assess the efficacy and safety of generic LMWH, orthopedic surgery appears to be a good model by using validated surrogate endpoints such as major bleedings and all deep vein thrombosis assessed by venography. The clinical relevance of this endpoint has to be discussed as well as the non inferiority margin.
In recent years, the separate nature of arterial and venous thrombotic events has been challenged. Indeed, several recent studies have consistently shown that patients with venous thromboembolism (VTE) are at a higher risk of cardiovascular diseases, including atherosclerotic complications, than matched control individuals. From the one hand, atherosclerosis has the potential to promote the development of thrombotic disorders in the venous system; on the other hand, the two clinical conditions may be simultaneously triggered by biological stimuli responsible for activating coagulation and inflammatory pathways in both the arterial and the venous system. Future studies are needed to clarify the nature of this association, to assess its extent, and to evaluate its implications for clinical practice.

**Summary**

In recent years, the separate nature of arterial and venous thrombotic events has been challenged. Indeed, several recent studies have consistently shown that patients with venous thromboembolism (VTE) are at a higher risk of cardiovascular diseases, including atherosclerotic complications, than matched control individuals. From the one hand, atherosclerosis has the potential to promote the development of thrombotic disorders in the venous system; on the other hand, the two clinical conditions may be simultaneously triggered by biological stimuli responsible for activating coagulation and inflammatory pathways in both the arterial and the venous system. Future studies are needed to clarify the nature of this association, to assess its extent, and to evaluate its implications for clinical practice.

**Association between venous thromboembolism and atherosclerosis**

Venous and arterial thrombotic disorders have long been viewed as separate pathophysiological entities, partly as a result of the obvious anatomical differences, as well as their distinct clinical presentations. However, a number of evidences suggest that this dichotomy is likely to be an oversimplification. Fibrin-rich thrombi form in the left atrial appendage of patients with atrial fibrillation and in the coronary artery system of patients with myocardial infarction (1). Likewise, platelets play an inevitable role in the formation of thrombi in the venous system (2,3).

The potential association between venous thromboembolism (VTE) and atherosclerosis was described for the first time in 2003 (4). Ultrasonography of the carotid arteries was performed in almost 300 unselected patients with deep vein thrombosis (DVT) without symptomatic atherosclerosis and in 150 control subjects. The presence of carotid plaques in patients with DVT of unknown origin was compared with that of patients with secondary DVT and that of control subjects. After adjusting for age and other risk factors of atherosclerosis, the odds ratio (OR) for carotid plaques in patients with unprovoked as compared to secondary DVT and controls was statistically significant.

**Key words**

Venous thromboembolism, deep vein thrombosis, pulmonary embolism, atherosclerosis, risk factors
In recent times, two studies have provided further evidence supporting the association between VTE and atherosclerosis. In a retrospective case-control study Hong et al found a higher prevalence of coronary artery calcium in patients with unprovoked VTE than in the control group (5). In a cohort of almost 30,000 consecutive autopsies Eliasson et al found an increased prevalence of VTE in patients with arterial thrombosis (6).

**Nature of the association**

On the one hand, atherosclerosis has the potential to promote the development of thrombotic disorders in the venous system. Atherosclerosis is associated with a detectable activation of both platelets and blood coagulation as well as an increased fibrin turnover, which can lead to thrombotic complications (7). On the other hand, the two clinical conditions may share common mechanisms or risk factors (such as old age, obesity, cigarette smoking, hypertension, hyperlipaemia, diabetes, and metabolic syndrome) (8).

**Is atherosclerosis predictive of VTE?**

In an attempt to assess whether atherosclerotic disease predisposes to VTE, the authors of two similar population-based cohort studies carried out in the U.S.A., the Atherosclerosis Risk in Communities (7) and the Cardiovascular Health Study (9), evaluated the rate of VTE development in subjects with prior subclinical atherosclerosis. In neither study were they able to show an association between subclinical parameters of atherosclerosis and subsequent VTE. Hence, subclinical parameters of atherosclerosis are unlikely to predict the occurrence of subsequent venous thromboembolic events.

**Is VTE predictive of arterial cardiovascular events?**

The incidence of arterial cardiovascular events after VTE was assessed in long-term prospective studies on the follow-up of patients with the first episode of pulmonary embolism (PE) or DVT (10,11). After adjusting for age and other risk factors of atherosclerosis, the idiopathic nature of the index VTE episode was confirmed to be an independent risk factor for arterial cardiovascular events at the long-term follow-up in both studies.

In a retrospective cohort study, Bova et al showed a higher rate of subsequent arterial events (acute myocardial infarction, ischemic stroke and peripheral arterial disease) in patients with spontaneous VTE than in control subjects randomly selected from the database of two family physicians. abnormalities of various blood constituents (12).

Recently, we performed a 20 year population-based cohort study using nationwide Danish medical databases (13). After excluding those with known cardiovascular disease, we examined the risk of hospitalization due to myocardial infarction and stroke among more than 25,000 patients with DVT, almost 17,000 patients with PE, and more than 160,000 population controls (13). Patients with both DVT and PE had a substantially increased risk of myocardial infarction and stroke during the first year after the thrombotic event. For patients with DVT, the relative risks varied from 1.60 for myocardial infarction to 2.19 for stroke. For patients with PE, the relative risks were 2.60 for myocardial infarction and 2.95 for stroke. The relative risks were also elevated, though less markedly, during the subsequent 20 years of follow-up, with 20 to 40% increases in risk for arterial cardiovascular events. Relative risks were similar for those with provoked and unprovoked deep venous thrombosis and pulmonary embolism.

**Conclusions and implications of the association**

Patients with VTE have a substantially increased long-term risk of subsequent arterial cardiovascular events. These findings have several implications for both research and medical practice.

Patients with VTE, particularly those of unknown origin, could be examined for asymptomatic atherosclerosis, in order to modify aggressively the risk profile in those with abnormal test results. Measures could include appropriate counseling about lifestyle changes and control of risk factors for atherosclerosis, as well as primary prophylaxis with antiplatelet therapy or statins.

In conclusion, the separate nature of arterial and venous disorders has been challenged. Future studies are needed to clarify the nature of this association, to assess its extent, and to evaluate its implications for clinical practice.

**References**


Venous and arterial thrombosis is a significant problem in the medical patient due both to its high prevalence and morbidity. In this presentation, the epidemiology of these events is presented, focusing mainly in deep venous thrombosis (DVT), arterial non-coronary thrombosis and cerebral thrombotic ischemia in medical patients. DVT incidence is estimated at 67 in 100,000, cerebral infarction (a cause of 60% of all strokes) occurs in 1 – 3 per 1000 individuals per year while accurate indices of prevalence can only be acquired via post-mortem examinations. Incidents rates become much higher with multiple risk factor clustering since thrombosis is a multicausal disease. Risk factors for DVT include age of the individual, sex, long periods of immobility, previous history of DVT, various hypercoagulable states, oral contraceptives and prolonged immobility which is especially significant for hospitalized patients. Of note, DVT is not unknown to happen to children, where in ages less than 1 year it is associated with venous catheters. In older adults, the much higher incident rate seems to derive from progressively larger numbers of risk factors clustering. Risk factors for arterial thrombosis and cerebral thrombotic events are generally different from those for DVT because they are mostly related to atherosclerosis. Thus, patients at high risk are smokers, those with hypertension, diabetes mellitus, obesity, hypercholesterolemia, various hypercoagulable states and females using oral contraceptives. Thrombosis is a preventable disease: incidence of arterial thrombosis can be reduced with risk factor management such as lifestyle changes with weight loss, lipid profile lowering, smoking cessation and optimal glucose control. Moreover, venous thrombosis can also be addressed with more active measures, including elastic undergarments, exercise of the calf and leg and early patient mobilization. In clinical settings, anticoagulants should be adequately administered according to patients' risk profile to achieve target INR.
When discussing thromboembolism, Virchow’s triad may still be used to simplify understanding of the pathophysiology. For a thrombotic process to start at least two of the three main components of the triad need to be altered: vessel wall, blood coagulability and blood flow. Those components are of interest both in arterial and venous thrombogenicity, although they may vary of pathogenetic importance. So in venous thrombosis decreased flow in combination with an activated coagulation seem important to initiate the process, whereas in arterial thrombosis vessel wall injury in the form of an arteriosclerotic ulceration in combination with activated platelets probably dominate. However, the processes are complex and various steps may be in common. To increase the understanding of the relation between arterial and venous thrombosis is important when discussing end points in studies on substances influencing hemostasis such as thromboprophylaxis. So for instance giving agents to decrease postoperative venous thromboembolism may on one hand also decrease the risk of arterial thromboembolic events but may on the other hand induce bleeding in the arteriosclerotic plaques with deleterious consequences. To contribute to an increased understanding we have analyzed the coexistence of arterial and venous thromboembolism in an autopsy series before the era of extensive use of thromboprophylactic substances. In a cohort of 23796 consecutive autopsies from 1970 to 1984 (84% of all in-hospital deaths) the prevalence of venous thromboembolism was related to the presence of arterial thrombosis. Arterial thrombosis was associated with 30% increased odds of venous thromboembolism and proximal deep vein thrombosis. The excess risk remained in the subgroups of cerebrovascular, visceral, iliofemoral and aortic thrombosis, also when controlling for age, gender and ischaemic heart disease as a cause of death in a logistic regression analysis. A negative association between venous thromboembolism and coronary thrombosis in the univariate analysis did not remain significant in the multivariate analysis. Thus, a positive association was found between arterial and venous thromboembolic disease except for coronary artery thrombosis, a possible competing cause of death.
Pathophysiology of Haemostasis and Thrombosis

SYM III.4: Pregnancy as a procoagulant trigger

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During pregnancy almost every organ of the mother’s body has to work harder in order to meet the demands of the developing fetus. As healthy pregnancy progresses, the mother is propelled into an increasingly proatherogenic metabolic syndrome. She develops a high cardiac output, venous stasis, hypercoagulability and increased inflammatory activity. After twenty weeks she develops insulin resistance and dyslipidaemia. It is known that the process of haemostasis is a dynamic equilibrium between the coagulation and fibrinolytic systems. During pregnancy the overall balance shifts towards a hypercoagulable state which is more marked around term and in the immediate post-partum period, returning to the non-pregnant state at approximately four weeks after delivery. The major changes in haemostasis factors during pregnancy in relation to the non-pregnant state are illustrated in the table.

Given that the levels of procoagulant factors increase during pregnancy, it is plausible that there is a higher risk of thrombosis during this period. The risk could be exacerbated by the presence of pathological conditions such as Antiphospholipid syndrome, Inherited thrombophilia, pre-eclampsia and thrombotic microangiopathies diseases.

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In 1975, Kline and associates published a prospective, blinded study on dextran vs saline in a mixed surgical population with autopsy proven fatal pulmonary embolism (PE) as the main outcome. They found a statistically significant reduction in fatal PE (1). Two years later, Johnson, Green and Charnley presented a 12 years material on venous thromboembolism (VTE) in total hip replacement (THR) patients. During the first part of their observation period, fatal PE was found in more than 2% and non-fatal events in about 15% of their operated patients. Based on this rather unpleasant finding from their own department, they started to use chemical thromboprophylaxis. However, it took several years with trying and failing to sort out a suitable regimen that not increased bleeding. Finally, they found an acceptable compound and dosing regimen, and at the end of their study period fatal and non-fatal PE had dropped to the half. At that time PE was the single largest cause of death following major orthopaedic surgery (2). Compounds with antithrombotic properties had been successfully introduced and proven their efficacy and safety. General prophylaxis was recommended.

Later, the scenario has changed. During the last two decades, the annual incidence of clinical deep vein thrombosis and pulmonary embolism decreased from around 5% to approximately 2% (3). Even the overall postoperative mortality, dominated by vascular thrombotic deaths have decreased in some regions and is now 0.2 – 0.4%. Unfortunately, lack of international standardization on how to calculate and give the figures on postoperative mortality makes published figures confusing. Mortality may be given relative to the death rate in the normal population, relative to a selected age matched group or as its own control and the figures differ (4-6).

Amazingly, no correlation has been found between several thromboprophylactic regimens applied for about one week (mainly started the evening before surgery), and the postoperative death rate. Thus, there may be several explanations for this decreased death rate during the last decades. One reason may be generally better health among people in many regions, since an increasing number of centenarians have been registered.

In a large epidemiological study, it was recently shown that the highest death rate was the day of surgery and that the vast majority died from vascular thrombotic events (7). This confirmed earlier studies from the sixties and seventies. The clotting process was triggered by the
trauma and in particular when tissues with high tissue factor (TF) content were damaged. Activated cells and cell fragments were released to the circulation and caused local haemostasis and even thrombus formation in damaged veins if the process overshot. In addition, cell aggregates and debris where brought with venous blood to the lungs where they caused cellular entrapment and triggered fibrin deposits (8-10). In animal studies, injection of TF caused microemboli aggregation in the lung vessels (and even other organs). This led to a high frequency respiratory distress and fatal outcome occurred. The effect of the TF triggered process was counteracted by injection of antithrombin (11,12). Later, ultrasonographic studies that visualized central veins showed showers of echogenic material consistent with cellular debris and cell aggregates that passed by in central venous blood, mainly during damage of the bone marrow (13). These procoagulant cells and fragments triggered a substantial clotting activity in the lung vessels reflected in significantly higher FPA and TAT values in arterial blood leaving the lungs as opposed to in mixed venous blood entering the lungs (14, 15). During surgery this caused in between a drop in PaO2 and triggered cardiorespiratory and vascular dysfunction.

Sequestered cells, microembolic aggregates and particles may pass in to the arterial circulation through several ways i.e. from entrapped cells in lung vessels, through lung-shunts or directly through a patent foramen ovale and cause thrombus formation at any cite of reduced function e.g. pathological flow, stenosis (e.g. atherosclerosis), stasis and vessel wall defects (15 - 18). Parallel to this systemic procoagulant activity the surgical trauma releases vasodilating substances that may induce reduced blood flow for many weeks after the operation both in the operated and non-operated limb (19).

Thus, new knowledge has made us change focus from solely postoperative venous thrombosis to include even arterial events. Thromboses may become clinically apparent from the time of surgery to several months later and manifest at any site of the vascular tree depending on the magnitude and dissemination of the clots. Hip fracture patients at high age that often are comorbide are in particular predisposed to such complications and seem to roll over in a second phase of increased procoagulant mortality that persists life-long (5). Recently a large epidemiological study showed an increased risk of arterial thrombotic events up to 20 years after the first episode of postoperative venous thromboembolism, confirming previous data (20).

A recent analysis based on studies with high autopsy rates and large cohorts, showed that thromboprophylaxis reduced overall mortality in the same proportion as fatal pulmonary embolism, i.e. approximately 50%. This indicates a protective effect even on the arterial side with the same antithrombotic regimens used to prevent venous thrombosis (21).

Taken together. Orthopaedic surgery causes a tremendous damage to the bone marrow. This triggers a substantial local and systemic thrombin generation and activity. Depending on the focus that favour thrombus formation, symptoms may manifest at any cite of the vascular tree. Vascular death is the main cause of postoperative mortality. The highest death rate is the day of the operation and it may continue for up to 3 months. Old and comorbide patients are rolling over in a second postoperative phase of sustained increased vascular mortality. We are about to enter a new era - a paradigm shift - moving from focus on postoperative venous thrombosis to systemic vascular thrombosis.

References

Several statin-based trials (e.g. 4S, CARE, LIPID, GREACE, HPS, TNT) involving patients with coronary heart disease (CHD) showed that these drugs significantly reduce the number of strokes. In TNT treating CHD patients with atorvastatin 80 mg/day was more effective than using atorvastatin 10 mg/day. SPARCL confirmed that aggressive lipid lowering with atorvastatin was effective in preventing both cerebrovascular and cardiac events. A key feature of SPARCL is that the patients had previous cerebrovascular events but no overt CHD. Statin use may also improve the outcome following open carotid surgery or endovascular procedures and even result in regression of carotid artery atherosclerosis. The mechanism responsible for these beneficial effects may involve plaque stabilization/regression in the carotid artery and aortic arch.

There is ample evidence showing that antiplatelet agents significantly lower the risk of stroke. The question is which drugs to administer and whether to use combination therapy. "Too much" inhibition may be associated with an increased risk of haemorrhagic strokes.

Hypertension is the most powerful modifiable predictor of stroke. Clearly, this problem needs to be addressed. It is still not established if some blood pressure lowering drugs are more effective than others in the prevention of stroke.

Diabetes is another predictor of stroke. It is not clear if good glycaemic control results in a significant reduction of strokes or in regression of carotid atherosclerosis. However, good glycaemic control is essential for the prevention of microvascular complications.

Smoking is a powerful vascular risk factor. We need a greater effort and more pharmacological options to address this problem.

The role of modifying "emerging" risk factors (e.g. homocysteine, lipoprotein, C-reactive protein and fibrinogen) remains to be established.

Modifying these risk factors will also decrease CHD-related events since the patients with carotid artery stenosis are at high risk of such events.

More attention needs to be paid to preventing strokes and TIAs.
Two recent randomized clinical trials comparing carotid artery stenting (CAS) vs. carotid endarterectomy (CEA) in symptomatic patients have been published. The EVA3S trial demonstrated that CAS has higher rates of death and stroke at 1 and 6 months compared to CEA (9.6% vs. 3.9% at 1 month, 11.7% vs. 6.1% at 6 months, respectively). The SPACE study failed to prove non-inferiority of CAS compared with CEA for the peri-procedural (30 days) complication rate (6.84% vs. 6.34%). These data do not justify the widespread use of CAS for treatment of carotid artery stenoses.

The results of these studies are different from those of the SAPPHIRE, that demonstrated that CAS with the use of an emboli-protection device is not inferior to carotid endarterectomy. The SAPPHIRE included both asymptomatic and symptomatic patients, at high risk due to medical or anatomical factors. EVA3S and SPACE included only symptomatics.

What factor can explain such a big difference between the European and the US studies? In the SAPPHIRE, 27% of patients had received a previous CEA, 29% a previous angioplasty; recurrent stenosis may bias results in favor of stenting because redo endarterectomy is associated with an increased risk of nerve lesions and stroke, while CAS is less risky with restenotic lesions due to the presence of intimal hyperplasia, which is associated to a lower embolic load to the brain. On the other side, the EVA3S and SPACE trials excluded restenoses. This is a huge difference, considering that carotid plaque morphology and echogenicity of restenoses (fibrotic tissue) is completely different from those of primary lesions (lipidic and hemorrhagic tissue). Carotid plaque morphology could be one of the factors explaining the different results of the above-mentioned studies.

The Imaging in Carotid Angioplasty and Risk of Stroke (ICAROS) Study analyzed the role of carotid plaque morphology (evaluated by the Gray Scale Median, GSM) as a predictor of stroke following CAS. The GSM is a computer-assisted grading of the echogenicity of carotid plaques. It is a measure of the overall plaque echogenicity, which is a quantitative index of the echoes registered from the plaque. The GSM has two main advantages: it’s easily and readily comparable and is calculated using a computer. The ICAROS Study demonstrated that the clinical impact of GSM relies on the ability to identify a wide number of patients (the prevalence of a GSM value less than 25 was 37% [155/418 Pts]) at higher risk of stroke during CAS (7.1 vs. 1.5%, p<0.005; OR 7.11) and to distinguish subsets of patients (with restenosis or with protected procedure) in which the
rate of neurological complications is different from the overall population. The logistic regression model confirmed the role of GSM as a predictor of stroke in both NASCET/ACAS-eligible and -ineligible patients. As a consequence of this, the effectiveness of GSM can be evaluated both in low (such as in CREST) and high-risk (such as in SAPPHIRE, ARCHER) patients.

Another issue deserves great consideration. CAS is associated with new areas of cerebral ischemia, as detected by using Diffusion-Weighted MR Imaging (DW-MRI). Not all DW-MRI lesions showed after few days following the procedure are irreversible ischemic lesions, around 40% of early DW-MRI lesions are definite brain infarction on follow-up MRI. Moreover, there is a correlation between the number of lesions in DWI as well as the volume of the lesions and the occurrence of brain infarction on follow-up MRI. The impact of these silent lesions was demonstrated in several studies. The most impressive finding is that the presence of silent brain infarcts at base line more than doubled the risk of dementia. Brain protection devices (BPDs) significantly reduced the death and stroke rate, as demonstrated in a review including 2536 CAS procedures. The limitation of BPDs is that they do not protect from silent cerebral ischemia, the rate of DW-MRI lesions in neuroprotected CAS ranges from 23 to 43%. As there is a significant correlation between microembolic signal count measured by transcranial Doppler and new DWI lesions after carotid treatment, the reduction of embolic load to the brain during the CAS procedure is essential to reduce silent cerebral ischemia after the procedure. Once again, low GSM value plaques generated a higher number of embolic particles during CAS. The selection of patients with low embolic potential to the brain may reduce the incidence of cognitive function.

In conclusion, only an appropriate indication to treatment will optimize the treatment for patients with carotid stenoses. The inclusion of carotid plaque morphology with the GSM calculation has the potential to reduce the risk of stroke during CAS and that of neurocognitive dysfunction months or years following the endovascular procedure.

References

Pathophysiology of Haemostasis and Thrombosis

SYM IV.4: Invasive treatment for carotid artery stenosis

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Based on the NASCET and ECST trials, surgery (carotid endarterectomy, CEA), is able to reduce the relative risk of disabling stroke or death by 48 % in symptomatic patients with severe stenosis (NASCET 70%, ECST 80%), and by 27 % with less severe stenosis (50-69% and 70-79% respectively). In lower grade stenoses, patients were harmed by surgery.

An appropriate recommendation should therefore be to perform CEA in symptomatic patients without contraindications provided >80 % stenosis (ECST) or >70% stenosis (NASCET).

Recent AHA guidelines, however, recommend CEA all from a stenosis grade of 50%, provided a perioperative stroke or death rate of less than 6%.

When discussing CEA to treat asymptomatic stenosis, the risk for perioperative severe complications must be considered as extremely important. Present evidence speaks in favour of CEA in patients with an expected survival of >5 years, and a risk of perioperative stroke or death <3%. The lower grade of stenosis remains unclear, AHA guidelines claim the range 60-99%. Sex and age differences also remain unsolved.

Carotid artery stenting (CAS) has been the case for several trials, with CAVATAS and SAPPHERE concluding CAS as safe and effective as CEA.

Two recent trials SPACE and EVA 3S arrived at opposite conclusions, EVA 3S was stopped due to a significantly higher rate of stroke and death and SPACE failed to show any benefit compared to CEA.

CAS for symptomatic stenosis can therefore not be recommended outside randomized controlled trials. No trial has been performed including only asymptomatic patients, while the SAPPHERE trial included 70 % asymptomatic cases. Specific conclusions regarding this group of patients can therefore not be drawn.

Interestingly, the AHA guidelines recommend CAS in patients at high risk for CEA with a symptomatic stenosis >70%.

A recent metaanalysis (J Vasc Surg 2008; 47:343) concluded that CAS is associated with a higher stroke and death rate than CEA, the role of CAS is therefore unproven. For patients with a high surgical risk, the role of any intervention is unproven.

Besides what can be concluded from trials so far, one may discuss a potential benefit of CAS in case of treatment of restenosis, treatment of patients with previous radiation therapy of the neck or any other critical morphological issue.

Further trials are required in which inclusion criteria are such, that the CEA and the CAS group are fully comparable.
The role of vertebrate blood coagulation is to rapidly prevent the loss of body fluids following vascular injury without compromising blood flow through either the uninjured or damaged vessels. To achieve this the coagulation network is initiated and regulated by a complex network of interactions that are under the control of both positive and negative feedback loops that result in controlled fibrin deposition and platelet activation only at the site of injury.

Mammalian blood coagulation is initiated by exposure of factor VII (FVII) to cells expressing the integral membrane protein tissue factor (TF). Activation of FVII to the protease FVIIa results in the activation of factors IX (FIX) and X (FX) by the TF/FVIIa complex. In the absence of its activated cofactor factor Va (FVa) FXa generates only trace amounts of thrombin. Although insufficient to initiate significant fibrin polymerisation, trace amounts of thrombin formed in this ‘initiation’ stage of coagulation are able to back activate FV and factor VIII (FVIII) by limited proteolysis. In the ‘propagation’ phase of coagulation FVIIIa forms a complex with the FIXa and activates sufficient FXa that in complex with FVa leads to the explosive generation of thrombin during that ultimately leads to generation of a fibrin clot.

Anticoagulant molecules play key roles in preventing inappropriate initiation of coagulation as well as down-regulating thrombin generation at the site of injury. The initiation complex is rapidly inactivated by the inhibitor Tissue Factor Pathway Inhibitor (TFPI). Antithrombin (AT) inhibits the active serine proteases directly. Whereas activated protein C inhibits the coagulation network by inactivating the cofactors V and VIII.

This presentation describes the initiation and propagation of the response and how it is ultimately down-regulated to prevent widespread inappropriate blood coagulation.
Arterial thrombosis mainly occurs at the site of atherosclerotic plaque rupture or erosion, and is primarily driven by the formation of a platelet-rich clot under high shear stress. Thrombin generation and platelet activation are the two leading mechanisms of arterial thrombosis, which finally incorporate erythrocytes and leukocytes, stabilized by the formation of a fibrin network. Tissue factor (TF), the receptor for coagulation factor VII, is the principal initiator of the blood coagulation cascade. After binding and activation of FVII, the TF-activated VII (FVIIa) complex activates factor X, leading to generation of thrombin that mediates platelet activation and fibrin formation. The procoagulant activity of the TF-FVIIa-FXa complex is down-regulated by the tissue factor pathway inhibitor (TFPI), which is physiologically localized at the surface of endothelial cells. TF and TFPI are both present in atherosclerotic plaques. Several lines of evidence suggest that TF is an important trigger for arterial thrombosis at the site of platelet rupture or erosion. TF is abundant in the necrotic core, and in the lipid-rich zones. TF expression mainly takes place in macrophages and smooth muscle cells, and is increased in the presence of oxidized lipids. TF overexpression is also closely related to plaque inflammation. Accordingly, TF expression is stronger in unstable than in stable plaques.

TFPI colocalizes with TF in atherosclerotic plaques, although the main cellular source may differ. Whether TFPI may locally regulate TF-mediated procoagulant activity remains uncertain. Relative plaque TF and TFPI levels are highly variables, and these variations may modify plaque thrombogenicity. These variations are directly related to the lipid content of the plaques, and possibly modified by lipid-lowering drugs.

The leading mechanisms for TF-mediated arterial thrombosis at the surface of an active atherosclerotic plaque are complex and incompletely understood. TF activation availability of active TF at the surface of cells expressing TF physiologically requires several steps of deenrcryption and cell membrane modifications. Active TF requires to be associated to phosphatidyl-serine, on the surface of cell membrane, or after formation of phosphatidyl-rich microparticles. Such a process is induced by apoptosis, which takes place in atherosclerotic plaques under strong immune and inflammatory regulation. Cell is also regulated by shear stress variations. Rupture or erosion of an inflammatory plaque may thus allow contact between active TF and FVII, leading to FVIIa formation, activation of the coagulation cascade and TF activation. It was recently demonstrated that TF, primarily localized at the surface of the plaque,
may migrate through the growing thrombus, probably associated with microparticles, and platelet or leukocyte membranes, thus leading to sustained thrombin generation on the thrombus surface. Moreover, the presence of TF in the flowing blood has been demonstrated. Although the actual role of this so-called "blood borne tissue factor" remains debated, it could contribute under certain circumstances to thrombus formation and growth. Parallely, the role of circulating TFPI, which is mainly associated to lipid, remains poorly known. A full understanding of the functions of these essential actors of arterial thrombosis would probably allow new therapeutic approaches, in addition to treatments targeting platelet aggregation and FXa or thrombin activity.
Currently methods of anticoagulation are in need of improvement. Thus, while unfractionated or low molecular weight heparins (UFH, LMWH) and warfarin derivatives are generally effective in prevention and treatment of thromboembolic arterial and venous conditions they also have several shortcomings, including: often poor compliance, delivery and less than ideal efficacy and safety profiles. For these reasons new anticoagulants are being developed to inhibit blood coagulation at several steps. Inhibitors of the initiation phase of coagulation may have theoretical advantages because they do not allow amplification of the coagulation cascade. Since tissue factor (TF) plays a significant role in the initiation of coagulation and is also associated with many diseases such cancer, atherosclerosis and inflammation its inhibition has been a focus of intensive research. Agents that inhibit TF initiated phase of coagulation include recombinant tissue factor pathway inhibitor (rTFPI), nematode anticoagulant peptide (NAPc2), active site-blocked factor VIIa (Factor VIIai), a variety of small molecules inhibiting factor VIIa, RNA aptamers (an small RNA-oligonucleotide), and TF targeting antibodies. Of those rTFPI (tifacogin) was promising in pre-clinical animal testing, but offered no benefit in reducing all-cause mortality at 28 days in patients suffering from severe sepsis. Presently tifacogin is being evaluated in patients with severe community acquired pneumonia. NAPc2, an 85 amino acid anticoagulant protein binds to non-catalytic sites of factor X and Xa and blocks the activity of the TF/VIIa/Xa complex. NAPc2 was effective in prevention of venous thromboembolism after elective knee replacement, though when compared to historical controls it was not different in efficacy/safety than LMWH. This agent is also being tested in several clinical trials involving patients suffering from unstable angina, non-ST-myocardial infarction, coronary angioplasty and metastatic colon cancer. However, the long half-life of rNAPc2 (50 hours) may present a safety concern in some clinical settings. Recently development of rNAPc2 was suspended for all indications. An active site inhibited Factor VIIa (FVIIai) acts as a competitive inhibitor of TF-dependent activation of factors X and IX. While effective in pre-clinical testing, its clinical assessment as an antithrombotic agent has thus far been disappointing when compared to heparin, especially in myocardial infarction, angioplasty and other medical conditions. Lastly, a new inhibitor of FVII/VIIa was isolated from a combinatorial RNA library, as an active aptamer. This new high affinity, nuclease-resistant RNA ligand binds specifically (like monoclonal
antibodies) to factor VII/VIIa thus preventing assembly of a functional FVII/TF complex. The major advantage of this agent is that its anticoagulant activities could be fully reversed by a specific antidote. Although overall progress has been made in developing this group of anticoagulants many obstacles still remain, regarding antidote availability, side effects and cost-effectiveness of some of them. These agents also compete with new inhibitors of thrombin and Xa (rivaroxaban) for various cardiovascular indications. In addition, a fast emerging novel area of investigation involves coagulation-independent activities of coagulation factors (TF, FIIa, FXa) that play an important role in processes such as wound healing, inflammation, angiogenesis, mitogenesis and cell survival, signal transduction and cancer. Future studies will shed more light on the consequences of coagulation factors inactivation by these new anticoagulant agents.
There is a need for new anticoagulants as alternatives to heparin, low molecular weight heparins and the vitamin K antagonists. A number of new drugs targeting single coagulation factors including thrombin and Factor Xa are under investigation.

After the withdrawal of ximelagatran, dabigatran is now the furthest advanced oral, direct thrombin inhibitor, and an extensive phase III clinical trial programme (the RE-VOLUTION trials) has recently determined its efficacy and safety in the prevention of VTE in major orthopaedic surgery. Fondaparinux, a synthetic pentasaccharide is the first Factor Xa inhibitor to be extensively tested in practice. Fondaparinux is a subcutaneous, indirect Factor Xa inhibitor, is approved for the prevention and treatment of venous thromboembolism (VTE) and in some countries for the treatment of acute coronary syndromes. Idraparinux, a subcutaneous, long-acting, indirect Factor Xa inhibitor, is in development. Direct Factor Xa inhibitors in development that show clinical promise in various indications include rivaroxaban, apixaban, betrixaban, LY-517717, YM150, and DX-9065a and its derivative Du-176b. Of these rivaroxaban and apixaban are the furthest advanced. Rivaroxaban has a favourable efficacy and safety profile, relative to enoxaparin, for the prevention of VTE after major orthopaedic surgery as shown in the RECORD trials. The results of trials of rivaroxaban for the treatment of proximal deep vein thrombosis are expected soon. It is likely that these new anticoagulants will revolutionize oral anticoagulant therapy.
Hirudin and its derivatives opened up a new way of inhibiting the coagulation system by attacking thrombin in several ways. They were injectable compounds with narrow therapeutic windows and achieved limited market position.

Two years ago, the first oral direct thrombin inhibitor, ximelagatran was turned down by FDA due to its potential liver toxicity. Currently the interest is focusing on dabigatran that have achieved approval by EMEA and may be available in the market within shortly, primarily for prevention of venous thrombosis in patients undergoing hip and knee prosthesis operations.

Dabigatran etexilate is the oral prodrug that is transformed into its active form when it is absorbed in the intestinal tract. It has a half-life in blood of about 15 hours, has a rapid onset at steady state and ~80% is renal excreted. It does not interfere with the CYP450 system and it has a very wide therapeutic area. In clinical dose titration studies, a dose response for any bleeding was found and small skin and mucosa bleedings occurred at a very high dose of 600 mg pr day. Dabigatran does not need any monitoring in the main stream of patients. If monitoring should be requested in specific medical conditions, commonly used global tests like APTT and INR can be used.

Based on several orthopaedic studies, two doses where selected for phase III studies i.e. 150 mg and 220 mg once daily, starting with a half dose (75 mg or 110 mg) the day of surgery. The first dose can be administered within a wide time frame from immediately after surgery (without increasing bleeding) to several hours later and may be due to a slower absorption the day of surgery. This may be clinically beneficial from a logistic point of view. The approved duration of prophylaxis in orthopaedics is about one and a half week following knee replacement and 5 weeks following total hip replacement surgery. No signals of liver or cardio toxicity have emerged. For the time being, studies are going on in more than 34 000 patients i.e. treatment of VTE, secondary VTE recurrence prophylaxis, long-term prevention of stroke in AF patients and prevention of cardiovascular events in acute coronary syndrome. This seems to be the first oral compound in 60 years that will reach the market and for the first time without all the drawbacks of the coumarine derivatives. The main stream of the patients will manage themselves with a convenient once daily oral compound that needs no monitoring and is safe and effective.
SYM VI.3: Comparative responses of some clotting assays to Fondaparinux, Dabigatran and Rivaroxaban

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Background
Dabigatran is a direct anti-thrombin inhibitor. Fondaparinux and Rivaroxaban are indirect and direct inhibitors of FXa respectively. Laboratory monitoring is not recommended, and has not been used in clinical trials. However it seems of interest to investigate the response of usual clotting assays and some more recently developed tests, with the aim of comparing their mechanisms of action.

Protocol
We have compared the response of prothrombin time (PT), activated partial thromboplastin time (aPTT), ecarin clotting time (ECT) Heptest, prothrombinase induced clotting time (PICT) and thrombin generation test (TGT using the method according to Hemker) to Rivaroxaban, Dabigatran and Fondaparinux spiked at increasing concentrations in a pool of citrated normal platelet poor plasma (PPP).

Results
We observed a concentration dependent prolongation of PT with Rivaroxaban and Dabigatran, while Fondaparinux has no significant effect on PT. However results obtained with Rivaroxaban may vary according to the thromboplastin reagent used. When the results are expressed in INR, the heterogeneity is not reduced.

aPTT is only prolonged by Rivaroxaban and Dabigatran, but not by Fondaparinux, depending on the reagent used. However Dabigatran prolongs aPTT but a relative saturation of the effect is seen with increasing doses. Thus, the ECT could be preferred since its prolongation is dose-dependant over a wide range of concentrations.

Conventional methods for Heptest (incubation period: 120 seconds) and PICT (incubation period : 180 sec.) measurement give a paradoxical response with low concentrations of Rivaroxaban: the clotting time is shortened. Then, increasing concentrations prolong the clotting time in a dose-dependant manner. When an antithrombin immuno depleted plasma is used, this paradoxical result is suppressed (figure 1). More over a shorter incubation time period (0 sec, 30 sec. versus 180 sec. for PICT) allows to suppress the unexpected result. Dabigatran and Fondaparinux induce a dose-dependant prolongation of both Heptest and PICT.

The three anticoagulants influence the various parameters of TGT. Dabigatran has the greatest influence on the initiation phase (LT) but does not prolong the time to reach the Peak (TTP) once the propagation phase get
started (TTP – LT), in contrast to Rivaroxaban and Fondaparinux. Profiles of calibrated automated thrombogram (CAT) are similar for Fondaparinux and Rivaroxaban. Using molar concentrations to compare the effect of anticoagulants shows that Rivaroxaban seems to be more active than Fondaparinux and than Dabigatran, in decreasing the Peak. However, it is difficult to compare rivaroxaban and dabigatran directly in this assay as the response curves have different profiles, the former affects mostly the amplitude of the peak, the latter affects not only the amplitude but also delays the peak. The endogenous thrombin potential (ETP) decrease is similar for the three anticoagulants.

**Conclusion**

PT could be used for Rivaroxaban laboratory monitoring when required and a standardized method is necessary since results obtained with different thromboplastin reagents may differ.

For Dabigatran the ECT could be preferable to aPTT. PICT and Heptest could be used for Fondaparinux and Dabigatran with the conventional technique. However the technique has to be modified to measure Rivaroxaban and could be standardized using an incubation period of 0 or 30 seconds. A similar observation has been made with the Heptest.

Thrombin generation alterations are different for Dabigatran which is more active in prolonging LT than Fondaparinux. In contrast the anticoagulant effect on ETP decrease is similar for the three agents.

**Figure 1:** Clotting time obtained with Rivaroxaban in PICT

<table>
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<tr>
<th>TGT (Fluvoroxaban and Species reagents)</th>
<th>TPT</th>
<th>ETP</th>
<th>Peak</th>
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<tr>
<td>Rivaroxaban</td>
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<td>0.08 ± 0.04</td>
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<tr>
<td>Fondaparinux</td>
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<td>Dabigatran</td>
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<td>0.05 ± 0.02</td>
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**Table 1:** Amount required to double LT, TTP and decrease of 50% Peak, ETP and mVRI

**Figure 2:** Thrombograms of the three anticoagulants
In the Netherlands oral anticoagulant therapy with vitamin K antagonists for outpatients is monitored by anticoagulation clinics or thrombosis services. These clinics are regionally organized which means that all patients living in a certain area visit the same clinic, irrespective of the referring physician or hospital. Nurses play an important role in the Dutch system. They are specially trained in anticoagulant control. The nurses collect blood samples of the patients at out-patients facilities or when necessary they visit the patients at home. With every venepuncture a standardized short history is taken. Subsequently, the INR is assessed at the laboratory of the anticoagulation clinic. In combination with the medical information the INR is fed to a special computer program after which specialized physicians determine the dosage and the control period. The dosage is printed on a dosage list which the patient receives the next day by mail. In case of bleeding complications or excessive INR results the patients are phoned the same day for dose adjustments or administration of vitamin K.

The number of patients is increasing in recent years especially due to the recognition of the efficacy and safety of atrial fibrillation as an indication for oral anticoagulant therapy. In 2006 nearly 8000 patients were treated by the Leiden anticoagulation clinic and 148000 INRs were assessed. The growing demands make it necessary to increase the possibilities for anticoagulant control. Point-of-care testing by patients themselves and patient self-management form an attractive alternative to conventional anticoagulant control. Since the official introduction in the Netherlands in 2002 a substantial part

SYM VII.1: The significance of the hospital anticoagulation clinic

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anticoagulation clinics and for the patients, organizes conferences and postgraduate training, promotes research and publishes a newsletter. A central overall quality assessment program with audits and accreditation has been introduced by the Federation. Every anticoagulation clinic send each year a report to the Federation comprising all data on the number of treated patients, the indications for anticoagulant therapy, therapeutic quality control (the percentage of INRs within the target range, adjusted for the type of coumarin derivative that is used) and all bleeding complications. From all this information the Federation composes an annual medical report.

The number of patients is increasing in recent years especially due to the recognition of the efficacy and safety of atrial fibrillation as an indication for oral anticoagulant therapy. In 2006 nearly 8000 patients were treated by the Leiden anticoagulation clinic and 148000 INRs were assessed. The growing demands make it necessary to increase the possibilities for anticoagulant control. Point-of-care testing by patients themselves and patient self-management form an attractive alternative to conventional anticoagulant control. Since the official introduction in the Netherlands in 2002 a substantial part
of patients on anticoagulant therapy now performs self-testing or self-management. In the Leiden anticoagulation clinic a total of 660 patients or 8% of the patients are on self-management and the number is still increasing.

The Dutch system of anticoagulant control is a way to ensure a high quality of care and due to the regional organisation structure, the high number of patients treated and the computerised registration of all relevant data it offers the opportunity for research. In recent years a number of anticoagulation clinics in the Netherlands have participated in research on causes of venous and arterial thromboembolism, therapeutic quality control, assessing optimal target ranges, patient self-management, the assessment of risk factors for bleeding complications, interacting co-medication, improvement of stability of anticoagulant therapy, development of a new computer algorithm for dosage prescription and comparison of the various coumarin derivatives that are available.
World-wide increase in the use of warfarin and other oral anticoagulant drugs in recent years followed the publication of studies demonstrating its value in a widening spectrum of clinical disorders. Improved benefit/risk ratio has resulted from the increased use of lower dose vitamin-K antagonist administration, pioneered in UK, and the Netherlands combined with the introduction of the WHO International Normalized Ratio (INR) system. There is an increasing demand for medical, technical, nursing, and administrative staff in hospitals and clinics, and many countries are being overwhelmed by numbers of patients requiring regulation of anticoagulant dosage. Therefore, dose control based on a variety of computer programmes is consequently being introduced. Previous studies claiming benefit from computer dosage have depended solely on laboratory results, i.e. time in therapeutic range of INR, and they have not been sufficiently large to determine, whether improvement in INR controlled by computer assistance resulted in clinical benefit or whether computer dosage was as safe as that by the experienced medical staff. In the first randomized computer-assisted dosage study, performed within the framework of the European Concerted Action on Anticoagulation (ECAA), now termed European Action on Anticoagulation (EAA), we were able to demonstrate (Poller et al. Lancet 1998; 352: 1505-9) that one widely used computerized dosage programme (DAWN AC) improved INR control both during introduction and maintenance of oral anticoagulation compared with medical staff dosage at the same clinical centre.

The size of the study, i.e. of computer-assisted dosage, was not sufficiently large to draw any conclusions concerning the clinical safety of computer-assisted anticoagulant dosage compared with traditional manual dosage by medical staff.

Nevertheless, there was a potential saving in medical staff time in dosage and possible administrative benefits from introduction of computerized decision support systems (CDSS). Such support systems can also help to identify patients with inadequate INR control and in addition suggest intervals for the re-testing.

The reliability of CDSS has recently been evaluated in the European Action on Anticoagulation (EAA), under the EC "Quality of Life and Management Programme" entitled: "Cost-effectiveness of Computer-Assisted Anticoagulant Dosage", using the clinical endpoints of bleeding and thrombosis and not only surrogate endpoint of time in therapeutic range. This is the largest randomized clinical endpoint trial ever of computer-assisted oral anticoagulant dosage. Thirty-two centres...
with a known interest in anticoagulant treatment in 14 countries and funded by the EC were recruited. Patients at individual centres were randomized to manual (medical staff) dosage and to one of two commercial programmes, either to a new version of the PARMA Programme (Parma 5) or to the established DAWN AC Programme. The use of two different commercial computer programmes aimed to preserve the independence of the studies from the industry.

A total of 13219 patients participated; 6503 patients were randomized to medical staff, and 6716 to computer assisted dosage. Most importantly, clinical events were reduced in the computer arm, and significant reduction in clinical events was observed in patients with deep vein thrombosis/pulmonary embolism by CDSS. Finally, regarding safety and effectiveness there was no evidence therefore that the introduction of CDSS increases risk of clinical events compared with experienced medical staff dosage. Significant benefit in achieving target INR was also observed in all the clinical groups in terms of INR in the therapeutic range.
Since the introduction of point-of-care equipment for the measurement of INR, the number of patients under oral anticoagulation treatment, performing self-testing and self-treatment has enormously increased. The quality of this so-called patient self-management is driven by two important aspects: 1) the quality of treatment 2) the quality of measurement. Both aspects will be discussed during the presentation.

**Quality of treatment**

The question is whether patient self-management of oral anticoagulation treatment results in an improved quality of life. In most recent years a number of studies have been published addressing this question. Mostly the mean time within the therapeutic range is the major measure for the quality of treatment. In several studies it was shown that patient self-management results in a 5 – 12% increase of the time within the therapeutic range, while other studies do not show an improvement in comparison to standard control by anticoagulation clinics.

Furthermore no extra bleeding or thrombotic complications were observed in comparison to patient groups under standard control by anticoagulation clinics. In particular a study recently published by Gadisseur et al (1) will be discussed. In addition to the quality of treatment, the quality of life of patient self-management was studied. Patient self-management results for the patient in an increase in general treatment satisfaction and a decrease in distress, perceived daily hassles and strain on the social network. It was also shown that education has played an important role in the effect on the quality of life parameters. This was also true for the group of patients under routine care after education.

In general it can be concluded that patient self-management may have a positive effect on the quality of life. However it should be realised that with a further increase in the number of patients starting patient self-management it may be the case that patients less capable of patient self-management might be included. This emphasises the importance of good education and coaching.

**Quality of measurement**

Since patients have changed from a regular INR control by an anticoagulant clinic to self-testing using point-of-care (POC) monitors the question should be addressed of whether both measurements are comparable. Published data on direct comparison between INR measurements with a POC monitor and a standard laboratory method is limited. In addition the
data available is contradictory. Some studies show a good agreement (> 90%), while other studies show for particular monitors poor overall agreement (Kappa statistic < 0.70). In general, it can be observed that an INR > 3.0 gives higher deviation with the standard laboratory method. However, it should be realised that also with different thromboplastins applied in standard laboratory methods clinical significant differences in INR measurements may be observed.

Another question to be addressed is the necessity for proficiency testing for POC monitors. In a recent study by Kitchen et al it was shown that in each proficiency testing survey 10 – 11% of the participating centres using POC monitors obtained INR results which were > 15% different from the consensus value (2). The authors emphasise the necessity for regular external quality control for POC monitors. Recently a new approach for external quality control for the CoaguChek INR monitors was published (3). Here a set of 5 different certified control samples were used with an INR value covering the whole therapeutic range. In a field study in The Netherlands it was shown that in about 20% of the total number of monitors checked (n=523) at least one of the control sample results was outside the tolerance limits (4,5). Based on this experience a concept for quality control of CoaguChek monitors, the most applied POC monitor so far, has been developed. This proficiency testing programme has now been introduced in The Netherlands. This concept will be discussed in detail.

References

It has been demonstrated that activated coagulation pathways are risk factors of cardiovascular disease in the general population. Fibrinogen is the major determinant of plasma viscosity and it strongly affects haemostasis, blood rheology, platelet aggregation and endothelial function, predisposing to thrombosis and enhancing atherosclerosis. Fibrinogen and its degradation products have been shown to stimulate smooth muscle cell proliferation and migration and accompany the elevation of acute phase proteins as a response to the mild inflammation involved in the earliest stage of plaque formation. Thus, elevated fibrinogen levels do not represent a casual cardiovascular risk factor only, but a risk marker for early atherosclerosis as well.

Arterial hypertension is a major risk factor for an array of cardiovascular and related diseases and because of its wide prevalence, the WHO has listed it as the first cause of death worldwide. Hypertensives are usually burdened with a variety of additional diseases and risk factors and have commonly elevated fibrinogen levels. We studied plasma fibrinogen levels in 18000 consecutive patients with uncomplicated essential hypertension. Fibrinogen increased in parallel to age (r=0.275), systolic blood pressure (r=0.268) and obesity (r=0.190), while it was inversely related to diastolic blood pressure (r=-0.163) and eGFR (-0.166). It had higher values in women (320 vs 310 mg/dl), non-dippers (334 vs 306 mg/dl), dyslipidaemics (337 vs 310 mg/dl), patients with metabolic syndrome (339 vs 301 mg/dl), central obesity (328 vs 295 mg/dl) left ventricular hypertrophy (327 vs 304 mg/dl) and microalbuminuria (341 vs 308 mg/dl). Smokers had increased fibrinogen levels, followed by ex-smokers and non-smokers (333 vs 316 vs 305 mg/dl). According to glucose metabolism, fibrinogen levels increased progressively from normoglycaemics to patients with impaired fasting glucose, impaired glucose tolerance and type 2 diabetes mellitus (302 to 310 to 331 to 360 mg/dl). Increased fibrinogen was to increased homocystine values (13.6 vs 11.9 Ìmol/L) and inflammation indices, as hsCRP (2.16 vs 1.26 mg/L) and serum amyloid A (6.30 vs 4.79 mg/L).

High plasma fibrinogen values are an independent cardiovascular risk factor and marker, while in hypertensive patients elevated fibrinogen levels are accompanied with disease severity, target-organ damage, low-grade inflammation, smoking habits, obesity and metabolic disorders.
Pathophysiology of Haemostasis and Thrombosis

SYM VIII.2: Metabolic syndrome, and haemostatic disorders

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The MetS, a concurrence of abdominal fat, disturbed glucose and insulin metabolism, mild dyslipidemia (high triglycerides and low HDL cholesterol levels), mild inflammation, and hypertension has been strongly associated with subsequent development of type 2 diabetes and arterial disease.

The existence of a prothrombotic state is a more recently recognized component of the metabolic syndrome which could contribute to accelerated atherothrombosis.

Subjects with insulin resistance, type 2 diabetes, or other components of the metabolic syndrome demonstrate endothelial dysfunction, which is at least partly a result of decreased production or increased clearance of NO and thus impaired blood flow, with a proadhesive phenotype (increased expression of VCAM, ICAM, E selectin…) as well as a decreased regenerative potential of the endothelium.

In vitro studies have shown a number of anomalies in the platelet functions in subjects with the MS. These anomalies account for hypersensitivity of platelets to aggregants and hyposensitivity to antiaggregants and are thought to contribute to enhanced atherosclerosis via increased platelet activity at sites of vessel injury.

The MS syndrome also has features of a hypercoagulable state, consisting of increased levels of clotting factors produced by the liver (factor VII). Recently the highly vascularized adipose tissue has been proposed as a major source of tissue factor involved in the initiating step of coagulation. Its expression level is influenced by insulin and glucose illustrating a possible link between glucose homeostasis and thrombosis.

The delay to thrombolysis observed in obese subjects is the most documented anomaly described in the MS. It has been attributed to increased PAI-1 levels. PAI-1 is an acute phase protein and the main antagonist of plasminogen activators. Beyond its function as an antifibrinolytic molecule, PAI-1 participates in processes involving angiogenesis and wound healing. Ectopic fat depots may represent privileged sites of PAI-1 synthesis during the MS. Interestingly there is also increasing evidence that PAI-1–dependent mechanisms may contribute to the pathogenesis of obesity and type 2 diabetes mellitus.

These alterations are the consequences of complex interrelations between insulin resistance, inflammation and oxidative stress. Secretion products of fat tissues (adipokines) may also be involved through direct effects on the vascular and the circulating cells.

In conclusion the last two decades of vascular biology research have yielded much information on the biochemical and cell biology factors involved in the vascular risk associated with the MS and it is hoped that this will lead to the discovery of agents that directly target these mechanisms. It is plausible that PAI-1 inhibitors might serve both in the control of atherothrombosis and insulin resistance.
Pathophysiology of Haemostasis and Thrombosis

SYMPOSIUM IX:
Inflammation-infection-sepsis and coagulation system

SYM IX.1: Coagulation cascade in sepsis

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In the pathogenesis of sepsis inflammation and coagulation play a pivotal role. Increasing evidence points to an extensive cross-talk between these two systems, whereby inflammation not only leads to activation of coagulation, but coagulation also considerably affects inflammatory activity. The intricate relationship between inflammation and coagulation may not only be relevant for vascular atherothrombotic disease but has also major consequences for the pathogenesis of microvascular failure and subsequent multiple organ failure, as a result of severe infection and the associated systemic inflammatory response.

Molecular pathways that contribute to inflammation-induced activation of coagulation have been precisely identified. Pro-inflammatory cytokines and other mediators are capable of activating the coagulation system and downregulating important physiological anticoagulant pathways. Activation of the coagulation system and ensuing thrombin generation is dependent on an interleukin-6-induced expression of tissue factor on activated mononuclear cells and endothelial cells and is insufficiently counteracted by tissue factor pathway inhibitor. Simultaneously, endothelial-bound anticoagulant mechanisms, in particular the protein C system, is shut-off by pro-inflammatory cytokines. Tumor necrosis factor-α (TNF-α)-mediated downregulation of thrombomodulin on endothelial cells appears to be a key phenomenon in this respect. In addition, fibrin removal is severely inhibited, due to inactivation of the fibrinolytic system, caused by an upregulation of its main inhibitor, plasminogen activator inhibitor type 1 (PAI-1). Increased fibrin formation and impaired removal leads to (micro)vascular thrombosis, which may result in tissue ischemia and subsequent organ damage.

Coagulation activity in sepsis can be detected by sensitive laboratory tests, however, most of these tests are not readily available in a routine setting. A reliable diagnosis can also be made on the basis of a small series of routine lab tests that can be combined in a scoring algorithm (ISTH-DIC score). Prospective validation of this score shows promising results.

The cornerstone of the management of coagulation in sepsis is the specific and vigorous treatment of the underlying disorder. Strategies aimed at the inhibition of coagulation activation may theoretically be justified and have been found beneficial in experimental and initial clinical studies. Heparin may be an effective anticoagulant approach and was shown to have a small clinical benefit in patients with sepsis and DIC. Alternative strategies comprise restoration of physiological anticoagulant pathways, by means of the administration of antithrombin concentrate, (activated) protein C, or strategies involving (recombinant) thrombomodulin. In particular, restoring the function of the protein C system by administration of activated protein C, was shown to be of benefit in patients with sepsis and organ failure.
Patients with sepsis almost invariably show evidence for activation of the coagulation system. Coagulation activation and deposition of fibrin can be considered instrumental in containing inflammatory activity to the site of infection. However, inflammation-induced coagulation may be detrimental in those circumstances when the triggered blood coagulation system is insufficiently controlled, which can lead to the clinical syndrome of disseminated intravascular coagulation (DIC) and microvascular thrombosis. Although the majority of sepsis patients do not have clinical signs of DIC, patients with a laboratory diagnosis of this syndrome are known to have a worse outcome than patients with normal coagulation parameters. In addition, several clinical studies have suggested that sepsis-related DIC is associated with not only a high mortality but also organ dysfunction and that attenuation of coagulation may ameliorate organ failure in this condition. The pathogenesis of DIC involves activation of coagulation with concurrent impairment of the function of anticoagulant mechanisms and fibrinolysis, resulting in a net procoagulant state.

More recent evidence indicates that severe infection also results in a disturbed hemostatic balance at tissue level. This has especially been documented in the lung. Intravenous injection of endotoxin (a toxic component of the outer membrane of Gram-negative bacteria) causes activation of coagulation in the bronchoalveolar space of animals. Similarly, patients with peritonitis (without clinically evident lung injury or infection) show evidence of coagulation activation in their lungs. Moreover, pneumonia is associated with a local (i.e., at the site of the infection) disturbance of the hemostatic balance, which involves stimulation of coagulation and inhibition of anticoagulant and fibrinolytic pathways.

Tissue factor is regarded as the primary initiator of coagulation in sepsis. Tissue factor is constitutively expressed by different cell types in the extravascular compartment, including pericytes, smooth muscle cells and keratinocytes, which upon disruption of the vascular integrity will get into contact with the bloodstream. In the lung several cell types express tissue factor, including macrophages and respiratory epithelial cells. Inhibition of tissue factor strongly inhibits coagulation activation in the systemic as well as the pulmonary compartment in models of severe sepsis and pneumonia. Tissue factor may not only influence coagulation but also inflammation: the tissue factor-VIIa-Xa complex can activate so-called protease activated receptors (PARs, especially PAR1 and PAR2) which results in an array of proinflammatory effects.
Blood clotting is controlled by three major anticoagulant proteins: tissue factor pathway inhibitor, antithrombin and activated protein C (APC). Recombinant forms of these endogenous anticoagulants have been tested in clinical sepsis trials; of these only APC was found to reduce significantly 28-day mortality in patients with severe sepsis. The biological effects of APC are pleiotropic, and can be roughly divided in anticoagulant and cytoprotective effects, the latter of which are mediated by PAR1. All components of the Protein C system are expressed in the airways. Pneumonia is associated with a reduced activity of the Protein C system within the airways.

This lecture will focus on the role of tissue factor and APC in the regulation of coagulation during severe infection, with special emphasis on the function of these pathways in the lung.

Literature

Disseminated intravascular coagulopathy (DIC) is common in septic patients and is associated with higher morbidity and mortality rates. However, it remains unclear as to whether the presence of DIC is a pathophysiologic mechanism or just a marker; in particular, at autopsy, thrombo-embolic phenomenon or even microthrombi are rarely evident. One could argue that the best evidence for DIC as a deleterious phenomenon per se is the observation of a beneficial effect on outcome with activated protein C, a natural anticoagulant. An international multicenter study in adults with severe sepsis comparing use of activated protein C to placebo therapies found a reduction in 28 day mortality from 30.8% to 24.7% in adults. Although patients with platelet counts less than 30,000/mm³ were excluded from this study, patients with platelets counts <100,000/mm³ and elevated thrombin-antithrombin complexes, diagnostic of DIC, had an increased survival benefit. Recent evidence, however, suggests that activated protein C does not act only on coagulation but also on complex inflammatory pathways involving endothelial protein C receptors (EPCR). Indeed, the negative results from studies with other anticoagulant drugs, such as antithrombin and tissue factor pathway inhibitor, suggest that the beneficial effects seen with activated protein C may have been more related to its anti-inflammatory effects than to its anti-coagulant properties. Some observations have suggested that patients treated with heparin may have a better outcome, but in these studies, patients were not randomized for heparin and it is possible that patients who were doing better simply became better candidates for heparin administration; thus the evidence supporting a role for heparin is not very convincing. Nevertheless, drugs that target the coagulation system are still being tested in preclinical and clinical trials. Recombinant human soluble thrombomodulin (ART-123, Artisan Pharma, inc) is the most advanced of these in terms of clinical testing and phase III clinical trials in Japan led to the drug being licensed there earlier this year for use in patients with DIC. A multicenter phase 2B clinical trial, which aims to assess the effect of ART-123 on 28-day mortality in 800 adult patients with sepsis and DIC, is currently ongoing.
For all pediatric cases diagnosed with thrombosis, anticoagulation is the hallmark of therapy. Follow-up data for VTE recurrence in children are available from few reports, unfortunately the duration of follow-up is variable across studies. Current therapy guidelines for secondary prophylaxis and prevention will be discussed.

Either Unfractionated heparin (UFH) or low molecular weight heparin (LMWH) is used as initial prophylactic agent to prolong patency and prevent obstruction of central lines as well as for anti-thrombotic treatment. An increasing rate of off-label use of LMWH in children has been reported, showing that LMWHs offer important benefits to children with symptomatic thromboembolic events and poor venous access. Recurrent symptomatic thromboses under LMWH occur in approximately 4% of children treated for venous thrombosis, and in 7% of children treated for stroke; major bleed was documented in 3% of children with therapeutic target LMWH anti-Xa levels, whereas minor bleeding was reported in approximately 23% of children receiving either therapeutic or prophylactic doses, respectively.

Thrombolisis is reserved for children with arterial occlusion, massive pulmonary embolism (PE) and in case of life, organ or limb threatening situations albeit major contraindication should be considered before administration. It has been suggested that thrombolysis may reduce long term complications of VTE, such as post phlebitic syndrome occurrence. Among children with stroke thrombolysis, though not indicated for patients <18 years of age, is currently being administered with unclear benefit due to diversity of protocols, doses and timing of administration.

The potential utility of new antithrombotic drugs in pediatric patients is currently under investigation.

The role of thrombophilia in the pathogenesis and prognosis of pediatric thrombosis, affecting the issue of anticoagulant therapy and its duration for secondary prophylaxis, remains to be elucidated.
CNS thrombosis in childhood is emerging as a serious and increasingly recognized disorder. The results of research in adults have limited applicability to children with stroke due to fundamental age-related differences in the neurological, cerebrovascular and coagulation systems. International incidence rates for stroke range from 1.3 to 13 per 100,000 children per year (adults: 100/100,000). Around half of the cases are ischemic and frequently of embolic origin. The great majority of ischemic strokes are caused by a diminished supply of arterial blood to brain tissue, whereas venous occlusions are not as common as arterial strokes in the pediatric population. Stroke occurs more frequently in neonates and infants. It is more common in boys. Risk factors for CNS thrombosis include vascular, intravascular and embolic disorders (congenital heart disease, infections, malignancies, sickle cell anemia, SLE, traumas, inborn errors of metabolism etc). Various congenital or acquired thrombophilic factors are also considered to be involved in thrombus formation. Nevertheless, the cause of childhood stroke is still unknown in one-third or even in half of the cases. The clinical presentation of ischemic stroke in children varies according to age and the location of the infarct. In neonates and infants the symptoms include seizures, lethargy, hemiparesis or hemiplegia, and, additionally, in older children, severe headache, confusion, dizziness, vision difficulties or hearing problems. Perinatal stroke is recognized only retrospectively. Evidence of infarction is usually detected within the middle, posterior or anterior cerebral artery distribution. Venous sinus thrombosis is responsible for most venous strokes. Vein of Galen malformation is a rare but important cause of mortality in neonates and infants. Brain imaging techniques document the diagnosis of arterial or venous thrombosis. A thorough investigation for an underlying disease, a triggering event and thrombophilic markers is always needed. Although prognosis of pediatric CNS thrombosis is better than for adult stroke, it is associated with considerable lifelong cognitive and psychiatric morbidity, as well as motor disability.
Thromboembolism is still regarded a rare event in childhood. During the past years, however, it is increasingly recognized as having significant impact on mortality, chronic morbidity and the normal development of children which has led to an enhanced sensitivity towards considering such events in respective patients. During the last decade much progress has been made towards better understanding of the underlying reasons causing thromboembolism in children. A considerable number of acquired and hereditary thrombotic risk factors have been identified which may also have an impact on therapeutic decisions and prognosis concerning outcome and the risk of a second event. However, indications for therapeutic interventions like thrombolysis and prophylactic anticoagulation with respect to the different clinical conditions and their combination with other risk factors are not well defined yet. The increasing knowledge of exogenous and endogenous thrombophilic risk factors has initiated a number of studies to assess the impact of such factors with respect to their contribution to the thrombophilic state both individually but also in concert with other factors. Besides their relevance for a first thrombotic event, much of the interest is now targeting their importance for thrombotic relapses. Only such studies will give us an answer to questions concerning the indications for treatment, prophylaxis and its optimal duration.
Thrombophilic risk factors are common and can be found in 5% to 25% of Caucasian populations. Because pregnancy is an acquired hypercoagulable state, women having thrombophilia may present with clinical symptoms of vascular complications for the first time during gestation or at the postpartum period (Brenner B Blood 2004).

**Thrombophilia and fetal loss**

The risk for fetal loss is greater in homozygotes than in heterozygotes with FVL and in female siblings of thrombophilic women who have FVL.

A recent meta-analysis demonstrated that FVL is associated with early (OR 2.01, 95% CI, 1.13, 3.58) and late (OR 7.83, 95% CI, 2.83, 21.67) recurrent fetal loss (Rey E Lancet 2003). Fetal loss has also been associated with prothrombin mutation (PTM) but not with the methylenetetrahydrofolate reductase (MTHFR) TT polymorphism. Combined thrombophilic defects were documented in 31 (21%) of 145 women who experienced pregnancy loss, compared with 8 (5.5%) of 145 in control subjects. (Sarig G Fertil Steril 2002).

Differences in type of pregnancy loss (ie, primary or secondary, isolated or recurrent, consecutive or nonconsecutive) and timing (ie, first, second, or third trimester) may also influence the magnitude of these associations. Recently, Lissalde-Lavigne and colleagues (J Thromb Haemost 2005) reported findings from the NOHA First study. The multivariate analysis clearly demonstrate an overall association between unexplained first pregnancy loss and the two thrombophilic risks factors and PTM Factor V Leiden. As unexplained first pregnancy loss occurred in approximately 10% of gestations, the findings of this study may have significant clinical impact.

A recent study by Gris and colleagues (Blood 2004) demonstrated that in women who had thrombophilia and previous one pregnancy loss after 10 weeks’ gestation, enoxaparin at a dose of 40 mg daily resulted in a significantly better live birth rate compared with low-dose aspirin (86% versus 29%, respectively).

The differences were found in women who had FVL and factor II G20210A and in women who had protein S deficiency.

LIVE-ENOX multicenter, prospective, randomized study recently conducted in Israel (Brenner B J Thromb Haemost 2005) comparing two doses of enoxaparin, 40mg/d and 40 mg/every 12 hours, starting at 5 to 10 weeks of gestation, throughout pregnancy, and for 6 weeks postpartum to women who had thrombophilia and
pregnancy loss. Of the 180 women enrolled, live birth rate before the study was only 28%, but during the study, live birth rates were 84% for the 40 mg/d group and 78% for the 80 mg/d group. Late gestational complications decreased after enoxaparin treatment. LMWH prophylaxis during pregnancy enables modulation of systemic hemostatic parameters by way of inhibition of factor Xa and increases in plasmatic total and free TFPI levels and may also modulate TFPI levels at the placental level (Sarig G, Thromb Haemost 2005; Aharon A J Thromb Haemost 2005).

**Future perspectives**

The role of aspirin, if any, in the setting of thrombophilia and vascular gestational abnormalities remains to be confirmed. As complete thrombophilia work-up is currently elaborate and costly, screening tests are highly warranted. One such potential assay is the protein C global test, which in a preliminary study was found to be abnormal in most women who had recurrent fetal loss and could also identify women who have recurrent fetal loss who do not have any other thrombophilic defect (Sarig G Thromb Haemost 2002). A recent study in mice demonstrates that a fetomaternal cross-talk in the placental vascular bed may result in control of coagulation by trophoblast cells (Sood R Blood 2006) and preliminary studies in human presented at the recent ISTH Geneva meeting by the NOHA study group, suggest that maternal and paternal EPCR polymorphisms may contribute to risk of fetal loss.

Several prospective multicenter controlled studies are currently underway in women with thrombophilia and pregnancy loss.
Pulmonary thromboembolism is the major direct cause of maternal death in the UK. In addition to mortality and acute morbidity, these women are also at significant risk of future deep venous thrombosis (DVT), venous insufficiency and pulmonary hypertension. VTE is up to 10 times more common in pregnant women compared to non-pregnant women of the same age and the risk in the puerperium is around 4 fold higher than in pregnancy. Symptomatic DVT in pregnancy has significant differences from the non pregnant. It is usually left sided (85% versus 55% in the non pregnant) and ilio-femoral (72% ilio-femoral in pregnancy versus 9% in non pregnant). Furthermore, VTE can occur at any time in the antenatal period with around 50% of events occurring in the first 15 weeks of pregnancy. Previous thrombotic event(s), age, obesity, thrombophilia and operative delivery are arguably the most important risk factors. Treatment and prophylaxis of VTE requires the use of anticoagulants, which have special considerations in pregnancy. Warfarin is associated with teratogenesis and a significant risk of bleeding in utero, particularly at delivery. Unfractionated heparin has particular problems in pregnancy; allergic reactions, thrombocytopenia, and heparin-induced osteoporosis. Low molecular weight heparin’s (LMWH) have substantially fewer side effects particularly with regard to heparin induced thrombocytopenia and osteoporosis and are usually the treatment of choice for prevention and treatment of VTE in pregnancy. LMWH may be combined with graduated elastic compression stockings. Prophylaxis is warranted in several situations including women with a single previous VTE and an underlying thrombophilia, and in women with multiple previous VTE. Women undergoing caesarean section and vaginal delivery should also have a risk assessment for VTE.

Objective diagnosis is essential when VTE is suspected and duplex ultrasound venography and ventilation-perfusion lung scans are the first line investigations. However investigations such as CTPA are increasingly being used for PTE, although they are associated with significant radiation exposure to the maternal breast, CTPA is associated with an even lower dose of radiation to the fetus than VQ scans. LMWH is usually used to treat acute VTE in pregnancy. The patient should also wear GECS for 2 years after DVT to minimize the risk of venous insufficiency.

Thus, there are particular problems with regard to venous thromboembolic disease in pregnancy and its management, which present a challenge to the obstetric physician.
SYM XII.1: Antiphospholipid Syndrome – molecular, clinical and therapeutic aspects

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The antiphospholipid syndrome (APS) is characterised by recurrent pregnancy loss and/or arterial/venous thrombosis in presence of either circulating lupus anticoagulant (LA) and/or antibodies to cardiolipin or β2-glycoprotein I (β2GPI). This condition is one of the most common causes of acquired thrombophilia and stroke under the age of 50 years. Anti-β2GPI antibodies in this condition are a good predictor of subsequent thrombosis. Understanding the biology and the role of β2GPI in health and disease is critical in unravelling the complex pathogenic mechanisms in the APS. Autoantibodies to β2GPI from patients with APS have been shown to be pathogenic when infused into mice and rats. Cell surface receptors that β2GPI interacts with on platelets and endothelial cells may play a critical role in the interaction of β2GPI with anti-β2GPI antibodies found in patients with APS. Anti-β2GPI antibodies in the presence of β2GPI results in the activation of platelets with the release of thromboxane A2 production which is inhibited by anti-glycoprotein Ibα antibodies. The proposed mechanism is that anti-β2GPI crosslinks β2GPI which is bound to GPIbα the extracellular domain of the GPIb complex on platelets. Activation of platelets via APL-β2GPI interaction with GPIbα induces activation of PI3K/AKT pathway downstream of GPIbα. This may be an important pathway for the induction of platelet activation and thrombosis formation in patients with the APS. Current best practice to reduce the risk of recurrent thrombosis in APS is oral anticoagulation. There is controversy in the area of intensity and duration of this treatment. However, studies have shown that APS patients have a rethrombosis rate in approximately 30% of cases and mortality of 6-10% despite antithrombotic therapy. There are still a number of critical issues relating to type and duration of anticoagulation in these patients.
Many studies have shown that among the tests exploring the presence of antiphospholipid antibodies, LAC is the strongest risk factor for thrombosis. Therefore, in our opinion, the first screening test to detect the presence of antiphospholipid (aPL) antibodies should be a coagulation test. In an analysis of the literature published between 1988 through 2001, a clear association between LAC positivity and thrombosis (OR range 5.71-7.3) was shown (6). Although grouping different studies (retrospective, ambispective, prospective) may influence the quality of results, the strong association of LAC with thrombosis may suggest that this test is the only one to rely on for a diagnosis of APS. Analyzing the studies between 1988 through 2001 it was found that the number of significant associations between aCL antibodies and thrombosis were found in only 6 out of 13 studies and the number of significant associations between ab2GPI antibodies and thrombosis were 10 out of 13 (7). Association with thrombosis is thus not significant for aCL nor for ab2GPI antibodies. In a cohort study our group found a significant association with thrombosis for LAC and ab2GPI antibodies and no association with aCL titre of more than 40 GPL or MPL (8).

It is not clear from Sidney consensus conference if diagnosis of APS could be made performing a single test. If this the case, frequent false positive and to lesser extent false negative results can be obtained in each test and this aspect will be analyzed.

In our opinion all the three tests should be performed and patients classified according to their antiphospholipid antibody profile.
Antiphospholipid antibodies (aPL) constitute an heterogeneous group of antibodies recognizing either phospholipids or phospholipid-binding proteins. Antibodies recognizing phospholipid-binding proteins have been associated with recurrent thrombosis or pregnancy morbidity; this combination is known as Antiphospholipid Syndrome (APS). The way we detect these antibodies was critical in order to differentiate them in subgroups. Thus today aPL can be categorized in the following subgroups: 1) Anticardiolipin antibodies (aCL) detected by enzyme-linked immunosorbent assays (ELISAs) using as phospholipid antigen the negatively charged phospholipid “cardiolipin”; 2) Antibodies to a phospholipids-binding protein called β2 glycoprotein I (β2-GPI); 3) Antibodies recognizing mainly prothrombin (PT) which can be detected by coagulation assays and especially by a prolonged activated partial thromboplastin time (aPTT). Of note, aCL from patients with APS recognize complexes of cardiolipin with β2-GPI, the latter found in the bovine serum which is used to block the non-specific binding sites in ELISA. Therefore, the main plasma proteins recognized by aPL are in fact β2-GPI and PT.

Cross-linking of aPL with either β2-GPI or PT does not explain very well the thrombogenic ability of these antibodies. This is because both of the above proteins lack any intracellular domain; thus cross-linking with antibodies does not seem enough for signaling inside the endothelial cell or platelet in order to change the phenotypes to a precoagulant form. Therefore, researchers have undertaken elegant experiments to detect adaptor proteins of β2-GPI or PT which possess intracellular domains in order to transducer signals into the cells.

Antibodies to phospholipids recognizing apolipoprotein receptors on platelet surface

In the light of previous evidence that aPL activate platelets significant efforts were undertaken to understand the mechanism of this activation; platelet extracted proteins were immunoprecipitated with anti-β2-GPI antibodies and the precipitated proteins were analysed by a second antibody which recognized the apolipoprotein E receptor 2 variant (apoER2) which is a splice variant of apoER2 unique in platelets. Indeed, apoER2 induced signaling leads to platelet activation. The described methodology is based on fishing expedition since we hypothesized that apoER2 should be the target of these antibodies and we found this molecule...
using appropriate monoclonal antibodies for which exact specificity, binding affinity, sensitivity of detection, appropriate controls were not tested.

Antibodies to phospholipids recognizing CD40 molecule associated peptides

There is evidence that human umbilical vein endothelial cells (HUVEC) cultured in the presence of anti-β2-GPI antibodies obtain a precoagulant phenotype as detected by upregulation of adhesion molecules, secretion of proinflammatory cytokines and tissue factor expression. Although these phenomena have not been observed by all the researchers and/or for all the antibody preparation tested it probably reflects one function of these antibodies which seems to be parallel with the function of TNF related family of proteins. Furthermore, there are experiments which suggest that combination of interleukin I with CD40 ligant mimic the function of TNF on endothelial cells as far as the expression of tissue factor and adhesion molecules is concerned. Thus, we searched for homologies between β2-GPI which comes out as the major antigenic target of aPL antibodies and the TNF receptor family of proteins and found an homology between amino acid residues 7-13 of β2-GPI and 239-245 of CD40. The CD40 peptide corresponding the amino acids 239-245 of the CD40 molecule was synthesized and was used for the detection of antibodies in APS and SLE patients. In fact 61.5% of APS patient’s sera and 72.7% of SLE patient’s sera positive for antiB2GPI antibodies, recognize the CD40 peptide. Furthermore, affinity purified anti-CD40 peptide antibodies and affinity purified anti-B2GPI antibodies, recognized both CD40 peptide and B2GPI. Finally, as was shown with confocal microscopy antibodies against CD40 peptide and B2GPI recognize the same molecules onto the cell surfaces. These findings suggest that anti-B2GPI cross reacting with CD40 molecules could potentially activate several cell types, initiating a coagulation cascade.

Antibodies to phospholipids recognizing complexes between B2GPI and glycoprotein Ibα (GPIba) on platelet surface

According to a recent study by the research group of S. Krilis, using direct binding assays and inhibition experiments, was shown that B2GPI could bind to the GPIba receptor on the platelet surface. The anti-B2GPI antibody–B2GPI complex was able to activate platelets, as was detected by the production of TXB2 from platelets, and this effect was inhibited by anti-GPIba antibody. The authors suggest that B2GPI complex via the GPIba receptor may contribute to the procoagulant tendency of APS.

Anti-B2GPI antibodies potentiate the inhibitory effect of B2GPI on thrombin-mediated Factor Xa Generation

B2GPI tends to interact with thrombin via exosites I and II, and anti-B2GPI antibodies produced an inhibition of thrombin-mediated factor XI activation in the presence of B2GPI. The physiologic significance of this finding need to be further examined but it could comprise evidence for interfering of B2GPI with the generation of thrombin and the role of B2GPI-antiB2GPI complexes in the plasmatic coagulation.
**SYM XII.4: New targets for the treatment of thrombosis in the antiphospholipid syndrome**

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The current antithrombotic approach to patients with the antiphospholipid (PL) syndrome (APS) is being completed with immunomodulatory treatments. For umpteen years, this syndrome has been defined by the presence of antibodies (Ab) to PL and/or β2-glycoprotein I (β2GPI). Since then, new insights into its pathophysiology have been gleaned from murine models particularly by the Shoenfeld’s group, supplying the impetus for new trials. For example, plasma exchanges have thus been applied to thrombotic events in patients with molecules that are prothrombotic and/or antifibrinolytic. A number of preliminary data are available. The mechanisms remain to be deciphered, but high-dose intravenous immunoglobulin has also met promising results. These are possibly based on the binding of a fraction of IgG to the B cell-activating factor of the tumor necrosis factor family. Recently, interleukin-3 has also been tested and shown to abrogate all the manifestations of experimental APS. Even more recently, β2GPI has been successfully administered, as an attempt to induce oral tolerance. Finally, APS could be the archetype of B cell-induced autoimmune diseases, and new biotherapies been developed accordingly. These include direct (anti-CD20 or anti-CD22) or indirect (anti-CD154 or anti-CTL4) monoclonal Ab, particularly in those patients with resistant forms of APS, such as the catastrophic APS. In the light of sustained responses in anecdotal cases, there is a need for further evaluations, when thrombocytopenic patients present at risk of hemorrhage.
For decades coumarins have been the most commonly prescribed drugs for therapy and prophylaxis of thromboembolic conditions. Despite the limitation of their narrow therapeutic dosage window, the broad variation of intra- and inter-individual drug requirement, and the relatively high incidence of bleeding complications, prescriptions for coumarins are increasing due to the aging populations in industrialised countries. The identification of the molecular target of coumarins, VKORC1, has greatly improved the understanding of coumarin treatment and illuminated new perspectives for a safer and more individualized oral anticoagulation therapy. Mutations and SNPs within the translated and non-translated regions of the VKORC1 gene have been shown to cause coumarin resistance and sensitivity, respectively. Besides the known CYP2C9 variants that affect coumarin metabolism, the haplotype VKORC1*2 representing a frequent SNP within the VKORC1 promoter has been identified as a major determinant of coumarin sensitivity, reducing VKORC1 enzyme activity to 50% of wild type. Homozygous carriers of the VKORC1*2 allele are strongly predisposed to coumarin sensitivity. Using individualized dose adaptation, a significant reduction of bleeding complications can be expected, especially in the initial drug saturation phase. Furthermore, concomitant application of low dose vitamin K may significantly reduce intra-individual coumarin dose variation and, thus, may stabilize oral anticoagulation therapy. The use of new pharmacogenetics-based dosing schemes and the concomitant application of low-dose vitamin K with coumarins will decidedly influence the current practice of oral anticoagulation and greatly improve coumarin drug safety.
Anticoagulants, including unfractionated and low-molecular weight heparins, as well as heparinoids and some of the previously used plasma volume expanders belong to the most widely used drugs. Hypersensitivity reactions from these agents are uncommon. However, when present they may have a considerable impact on patient safety and treatment decisions. Early diagnosis of potentially life-threatening adverse events and identification of alternatives is clinically important.

The most common hypersensitivity reactions are erythematous plaques, occurring with a delay after subcutaneous application of heparins. Seldom they can turn into maculopapular exanthema. Other hypersensitivity reactions are rare but may be life-threatening, e.g. skin necrosis due to heparin-induced thrombocytopenia (HIT II). Resistance to heparins in terms of modified pharmacokinetic due to shortening of the elimination half-life has not been investigated for hypersensitivity skin reactions. Heparin resistance is usually present in HIT II due to accelerated consumption of heparins. Skin and provocation tests with immediate and late readings are the most reliable diagnostic tools for heparin-induced urticaria/anaphylaxis or heparin-induced delayed plaques. If necrosis from heparins is suspected, skin tests are contraindicated. In anaphylactic reactions caused by dextrans or hydroxethyl starch skin tests are useless. Most in vitro tests have a low sensitivity and are not generally available.

In some anticoagulant-associated hypersensitivity reactions detailed allergologic investigation may help to identify safe treatment alternatives. However, several tests may be needed, and the procedures are usually time-consuming. Testing for allergy is particularly useful in certain patient groups (e.g. pregnant women), in which alternative solutions can be restricted.

If hypersensitivity to heparins is diagnosed, changing to a pentasaccharide might be the solution because this is a product with the lowest probability for cross-reaction. In cases where intravenous anticoagulation is needed or in cases with HIT II, alternative products as danaparoid, hirudins or argatroban can be safely used.
Laboratory aspirin unresponsiveness ("resistance") has been reported over the last 30 years, ranging between 5-50%, applying different methods, among individuals with a variety of cardiovascular disorders. From early works it has been realized that different methods give different results, depending on the end point of the tests. Thus results of testing plasma and urinary thromboxane metabolites suggest a very low rate of laboratory resistance (LR) whereas platelet aggregation in response to arachidonic acid (AA) yields a relatively higher LR rate. Recently similar observation has been made regarding the response to clopidogrel as well. The main questions arising from these studies are: a) what are the mechanisms responsible for the LR phenomenon? b) does LR confer adverse clinical outcome? c) is there a way to overcome LR in an attempt to improve outcome? and d) what are the available methods suitable for testing aspirin and clopidogrel LR.

Potential mechanisms responsible for LR to clopidogrel include genetic variability (in the P2Y12 receptor and in the cyp 450) leading to incomplete inhibition of the receptor or to ineffective metabolism of the pro drug, as well as the acuity of the clinical state of ACS. High rate of aspirin LR has also been reported among patients with ACS, which was associated with acuity of the disease as well as with a residual arachidonic acid response, potentially due to ADP activation and may be other pathways as well. Thus we and others reported a higher rate of aspirin LR among patients during acute ACS events compared to patients at a stable state of the disease. Similar results were found among patients with stroke, where severity of the disease was correlated with aspirin LR rate. Around 20-30% of patients with STEMI were found by us and others with clopidogrel LR as well and some studies reported an increased rate of adverse CV events among these LR patients. Recent meta-analysis by several groups reported that indeed LR to both aspirin and clopidogrel is associated with adverse CV events, with OR of around 4 for aspirin LR and around 8 for clopidogrel LR. These observations and meta-analysis have set the stage for future studies that should address the major question of whether personalized adjustment of anti-platelet drug therapy will be associated with improved clinical outcome as well. Preliminary studies by us and others suggest that increasing the dose, as well as combination drug therapy may improve the patient's response to the drug. However to date there are no reports on the correlation between improved laboratory responsiveness to clinical outcome. Finally, different laboratory methods have been suggested as potential tests for monitoring patients under anti-platelet drug therapy. A major open question regarding these tests is the definition of cut-off values allowing to distinguish between response and no response. This question could potentially be answered only after comprehensive studies will establish correlation between different cut-off values to adverse events as well as between dos adjustment and improved clinical outcome.
Cancer patients with venous thromboembolism (VTE) have an increased incidence of VTE recurrences and anticoagulant-related bleeding complications compared with those without cancer. A number of variables (i.e., age, renal insufficiency, recent bleeding, clinical presentation of VTE, ...) have been associated with an adverse outcome in patients with VTE, but there is little information on the influence of the cancer characteristics (site, extent, time elapsed since diagnosis) on outcome. A risk assessment tool that were able to quantify an individual patient’s risk of complications while on anticoagulant therapy would be useful by helping clinicians in evaluating the benefits versus risks of anticoagulant therapy in clinical practice.

The RIETE Registry is an ongoing, international (Spain, France, Italy, Israel, Argentina), multicentre, prospective registry of consecutive patients presenting with symptomatic acute VTE confirmed by objective tests.

We constructed a clinical prediction rule to identify which cancer patients with VTE are at a higher risk for recurrent pulmonary embolism (PE) or major bleeding during the first 3 months of anticoagulant therapy.

Up to May 2007, 3805 cancer patients had been enrolled in RIETE. Of these, 90 (2.4%) developed recurrent PE, 156 (4.1%) had major bleeding during the study period. A risk score was calculated: the incidences of recurrent PE in patients with low-, intermediate- or high risk were: 0.6%, 2.5%, and 7.4%, respectively. The incidences of major bleeding in patients with low-, intermediate- or high risk were: 1.8%, 5.0%, and 15%, respectively.

A simple risk score based on easily available variables may identify cancer patients with VTE at low-, intermediate- or high risk for recurrent PE or major bleeding during the first 3 months of therapy.
It is widely accepted that the incidence of VTE is increased in patients with cancer. However, patients with cancer are a heterogeneous group due to differences in tumor types, differences in stages and differences in treatment modalities. In a recent population-based case-control study the correlation between the percentage of cases that died within one year and the one year incidence rate of VTE was high (r=0.81) and it explained 64% of the observed variation in the VTE incidence. Most VTE events occur in the first year after the diagnosis of cancer. Antineoplastic treatment affects the VTE incidence. Central venous catheters are associated with a five-fold increased VTE risk. Surgical treatment has already been acknowledged as a risk factor thirty years ago. Various non-surgical treatments increase the VTE risk as well. Chemotherapy increases the four-fold risk of VTE in cancer patients to a six-fold increased risk. In a retrospective study the absolute incidence of VTE in 206 patients receiving chemotherapy 10.9% annually. Incidences of VTE in patients with chemotherapy differ widely depending on tumor type and type of chemotherapy.

Angiogenesis inhibitors like bevacizumab, are the most recent antineoplastic drugs that increase the VTE risk in cancer patients. In conclusion, cancer patients have a higher risk of VTE depending on tumor type, tumor stage and treatment. However, it is difficult to establish the size of this increased risk for a particular patient.
Venous thromboembolism (VTE) is a frequent complication in cancer patients and represents an important cause of morbidity and mortality. It has been estimated that 1 in every 7 hospitalized cancer patients who die, do so from pulmonary embolism (PE). Of these patients, 60% have localized cancer or limited metastatic disease that would have allowed for longer survival in the absence of a fatal PE. The incidence of VTE in cancer patients has been described to be approximately 15%, with reported incidence rates ranging from 3.8% to 30.7%. However, it is likely to be much higher, because VTE is often asymptomatic or minimally symptomatic and, even when symptoms are present, they are often nonspecific or mistakenly attributed to the underlying malignancy. Especially in patients who have a poor life expectancy, preventing death from pulmonary embolism is the mainstay of treatment. Some patients with deep vein thrombosis develop recurrent venous thromboembolic or bleeding complications mainly during anticoagulant treatment. These complications may occur more frequently if these patients have concomitant cancer. From a prospective follow-up study arise that in thrombosis patients those with cancer have a higher risk (20.7% or a hazard ratio of 3.2) for recurrent venous thromboembolism or bleeding during anticoagulant treatment than those without cancer (6.8%). In the same study, the cumulative incidence of major bleeding was 12.4% in patients with cancer and 4.9% in patients without cancer, with a risk ratio of 2.2. Recurrence and bleeding were both related to cancer severity and occurred predominantly during the first month of anticoagulant therapy but could not be explained by sub- or overanticoagulation. So, patients with DVT who also have cancer seem to be at a higher risk for recurrent venous thromboembolic complications during anticoagulation. In conclusion, Cancer patients with venous thrombosis are more likely to develop recurrent thromboembolic complications and major bleeding during anticoagulant treatment than those without malignancy. These risks correlate with the extent of cancer. Possibilities for improvement using the current paradigms of anticoagulation seem limited and new treatment strategies should be developed. During anticoagulant therapy, cancer patients have a 2- to 4-fold higher risk of recurrent VTE and major bleeding complications when compared with non cancer patients. The long-term administration of LMWH should be considered as an alternative to anti-vitamin K drugs in patients with advanced disease and in those with conditions limiting the use of oral anticoagulants. Prolongation of anticoagulation should be considered for as long as the malignant disorder is active. The evidence of lowered cancer mortality in patients on LMWH has stimulated renewed interest in these agents as antineoplastic drugs and raises the distinct possibility that cancer and thrombosis share common mechanisms.

The most common situations that increase the thromboembolic risk in cancer patients include immobilization, surgery, chemotherapy with or without adjuvant hormone therapy, and the insertion of central venous catheters.
Thrombin generation (TG) is a function test of the haemostatic-thrombotic system and a useful complement to information on single factors and genes. It is conveniently measured by conversion of an added fluorogenic substrate. It is useful where traditional tests give no, uncertain or ambiguous results such as the monitoring of factor VIII-inhibitor bypassing therapy, the discrepancy between clotting factor levels and bleeding tendency in haemophilia and rare coagulation disorders, measuring the effect of mixed treatment (OAC/heparin/antiplatelet therapy). Thrombin generation is increased in patients at risk for venous thrombosis due to congenital inhibitor deficiencies, through the use of oral contraceptives or lupus erythematoses as well as in patients with a tendency to recurrent venous thromboembolism.

Using fluorescent substrates raises the problem of how to relate velocities to thrombin concentration. This is no trivial problem because fluorescence intensity is not only dependent upon the concentration of the fluorophore but also upon the properties of the plasma in which the fluorophore is present. Furthermore, during the experiment the reaction velocity per unit enzyme decreases because substrate is consumed and the product concentration is not linear with the fluorescent signal. This can be compensated for by continuous individual calibration (CIC) in which the activity of a fixed known amount of calibrator (α2M-Thrombin) is measured in a parallel sample of the plasma under investigation over the entire course of the experiment. It will be shown that for clinical application the precision brought by adequate calibration is a conditio sine qua non.
SYM XV.2: Diagnostic value of D-dimer testing for venous thromboembolism and duration of anticoagulation

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Patients with a previous episode of venous thromboembolism (VTE) have a relatively high risk for recurrent VTE. The risk of recurrence is not the same in all patients and clinical and personal factors allow a stratification of patients into groups with increasing risk of VTE recurrence after discontinuation of anticoagulation. Patients whose index event was triggered by reversible major risk factors have a low risk and deserve a short treatment period; on the contrary, an indefinite anticoagulation is recommended in patients with active malignancy or other highly predisposing personal conditions due to their very high risk of recurrence. Still uncertain remains the optimal duration of treatment in patient with an idiopathic (unprovoked) event, whose rate of recurrence is estimated to range between 15% and 20% during the first 2 years after anticoagulation is stopped. A major problem lies in the difficulty of predicting the balance between the risk of recurrence after anticoagulation is stopped and the risk of bleeding due to a prolonged anticoagulation in individual patients.

D-dimer, a product of lysis of stabilized fibrin-clot, is a marker of coagulation activation. Its levels tend to increase in some patients with a previous VTE episode after anticoagulation is stopped. Several prospective studies have consensually demonstrated that the rate of subsequent VTE recurrence is higher in those patients in whom D-dimer levels increase after anticoagulation is stopped. It can be said, therefore, that the D-dimer levels measured after anticoagulation is stopped can be used as a tool to stratify the individual risk of recurrence.

A recent study tackled the problem whether or not D-dimer levels may also be useful to drive our decision on duration of anticoagulation in individual patients. The collaborative, prospective, randomized PROLONG study (N Engl J Med 2006;355:1780) investigated 608 patients with a first unprovoked venous thromboembolism who had received oral anticoagulation for at least 3 months. D-dimer testing was performed with the same qualitative assay in all participating centers one month after anticoagulation withdrawal. Patients with normal D-dimer (n. 385) did not resume anticoagulation. Patients with abnormal D-dimer were randomized to resume (n. 103) or not resume (n. 120) anticoagulation. All patients were followed for an average of 1.4 years. Study outcomes occurred in 6.2% of patients with normal D-dimer, and in 15.0% and 2.9% of those with abnormal D-dimer who were allocated to stop or to resume anticoagulation, respectively.

Preliminary results from a currently running, collaborative, observational, prospective study, which involves several Italian centers, show that patients whose D-dimer levels become persistently altered later than one month after anticoagulation withdrawal have also a very high risk of VTE recurrence and likely benefit from resumption of treatment.

The available data confirm that D-dimer assay, carried out after anticoagulation withdrawal, is an useful tool to assess the risk of recurrence and to regulate the duration of anticoagulation in individual patients with a first idiopathic VTE event.
The use of the thromboelastography to monitor whole-blood coagulation was first described by Hartert in 1948. This technique provided information on the initiation of coagulation, propagation kinetics, fibrin-platelet interaction, clot firmness and fibrinolysis. By miniaturization of the technology for the thromboelastograph (TEG®; Haemoscope Corporation, IL, USA) and the use of standardized reagents containing activator for the modified rotation thrombelastogram analyzer (ROTEM®; Pentapharm, Munich, Germany), they have been developed for a bedside use. Depending on the parameters measured, results are available within 30 minutes.

In orthotopic liver transplantation, one of the first clinical applications, TEG® was enable to detect enhanced fibrinolysis which is the most striking abnormality occurring in the late anhepatic stage. Moreover, the contribution of heparin to the reperfusion coagulopathy has been shown using TEG analysis.

In cardiac surgery, only post bypass TEG parameters have been shown to correlate with the risk of postoperative bleeding. But several authors have demonstrated that the TEG / ROTEM could be useful to guide the administration of blood products during cardiopulmonary bypass, and also using heparinase containing reagents, it could contribute to the assessment of coagulopathy versus the heparin effects.

We have recently shown that ROTEM® could detect early systemic changes in in vivo coagulation in trauma patients. A coagulopathy could be observed in almost 30% of these patients. Moreover, in 6% of trauma patients, ROTEM® could detect hyperfibrinolysis more quickly (≤ 15 minutes) and accurately than standard coagulation tests. As previously demonstrated in cardiovascular surgery, or in liver transplantation, “bedside” analysis of TEG / ROTEM® could help in trauma patients to guide a selective substitution management or to guide which patients could benefit from treatment with antifibrinolytic agents.

ROTEM® could also be useful in the management of patients with severe FXIII deficiency particularly in patients receiving prophylaxis. Current chromogenic methods lack accuracy for low FXIII levels. In our experience, ROTEM® was able to detect viscoelastic changes of fibrin clot in whole blood samples with low FXIII and was successfully used to monitor FXIII replacement therapy.

Other clinical applications of the test have recently been reported. Changes of TEG parameters during normal pregnancy were observed and preliminary data on the assessment of hypercoagulability using TEG suggested that thromboelastography could be a useful tool in the investigation of women with a history of recurrent miscarriages. In addition, hypercoagulability detected by thromboelastography in pancreas-kidney transplantation recipient has been recently reported and TEG analysis could be of interest in individually tailoring of anticoagulation therapy.
Heparin induced thrombocytopenia has received considerable attention during the last decade. An unknown or little known syndrome 20 years ago, it is today perhaps too common.

The first problem with “Advances in diagnosis and treatment of HIT” concern the exact definition of the condition. Thrombocytopenia does not mean HIT. Furthermore, positivity of anti-heparin/PF4 complex does not mean HITT.

The proportion of positive antibodies in the ELISA test found in patients suspected to have HIT is 1 out 20 samples. A tremendous waste.

This clearly indicates that the pre-test evaluation is lacking or faulting. There is today an urgent need for a set of rules which could, once applied, overcome the huge discrepancy between a platelet fall and the need for an antiplatelet antibody assay. This could be achieved by a pre-test selection of patients. It is known that orthopaedic patients and patients undergone cardiac surgery present a high incidence of HIT. In this field a significant advance has been supplied the confirmation of the validity of the 4T approach.

On the other hand, medical patients both admitted or in an out-patient clinic have only about a 1% incidence of HIT. There is an urgent need that caring physicians become expert in excluding other causes of thrombocytopenia in surgical patients, namely hemodilution, bone marrow failure, hypersplenism, mechanical consumption because of foreign surfaces.

Another aspect which could be of great help in clinical practice would be a test or a set of tests which could foresee the transformation of HIT in HITT.

A higher antibody titre is important but this is not always the case. Several patients have been reported in whom relatively high titres have shown no thrombotic effect.

Another problem to be resolved is that pertaining to the persistence of antibodies in post-HIT or post-HITT. Does this persistent positive titres have any significance?

Because of the discrepancy existing between ELISA and serotonin release assay (SRA) it would be important to develop a non radioactive isotope dependent serotonin release assay. Cytofluorimetric assay and the particle gel immunoassay have been shown to be reliable and to correlate well with the SRA. Finally, a recent problem in diagnosis is the definitive evaluation of the slide tests which have drawn, for this practical advantages, considerable attention a few years ago, but seen now to have last at least part of their glamour. Finally, do pre-existing variation in ATIII, fibrinogen or other parameters have any relation with the evolution of HIT into HITT? It would be very useful if this could be demonstrated.
As far as management is concerned, the major problem is still the choice of drug. Recent studies concern mainly the comparison between fondaparinux (an anti-FXa synthetic compound) vs direct thrombin inhibitors (DTI). The problem is still unsettled. The choice is important since, recently, a relation between these two groups of drugs and the initiation of coumarin medication (coumarin bridging) has emerged. Studies with limited numbers of patients seem to suggest that Fondaparinux might facilitate the coumarin bridging. Therefore a DTI – fondaparinux – coumarin sequence of events is proposed versus a direct DTI – Coumarin bridging. The therapeutic plot really thickens.

New tools of investigation and advance in management should concentrate in a prophylactic approach for patients known to be potentially predisposed to HIT or HITT who are to be treated with UF-heparin or LMWH. Do such drugs exist? Could fondaparinux alone or in combination with steroids or immunosuppressive patient play a role in such attempt?

The role of plasmapheresis in the treatment remain also to be defined. In medicine, as in life, the solution of a problem originates other problems.
Heparin-induced Thrombocytopenia (HIT) is a rare but potentially severe drug-induced immune adverse reaction occurring in 0.5 to 3% of cases. Heparin which is an antithrombotic gold standard is able to induce a major prothrombotic syndrome. For this paradoxical syndrome, the major problem is its recognition and its management which should be as earlier as possible to avoid the development of life-threatening complications.

HIT seems more often reported in inflammatory diseases or sepsis and surgical patients, requiring a greater awareness in these contexts. HIT was initially described fifty years ago by RE Weismann a vascular surgeon reporting recurrent thrombosis with curative unfractionated heparin leading to an iterative arterial white clot syndrome requiring amputation. The immune origin was established in the eighties. More recently, in 1992, J. Amiral, a French researcher, discovered the major target of HIT antibodies which is a macromolecular complex associating heparin and its natural plasma inhibitor released from platelet granules, the platelet factor 4 (PF4). In some cases, modified PF4 can be replaced by other chemokines such as Interleukin 8 or Neutrophil Activating Peptide 2. These antibodies induce a systemic and multicellular activation of the vascular compartment leading to a major acquired hypercoagulable state. Due to this increased thrombin generation, arterial and mainly venous thrombosis are frequently associated (>50% of cases) with the platelet count fall. Others unusual thrombotic episodes are reported such as bilateral adrenal haemorrhagic infarct, venous limb gangrene or skin necrosis. So, HIT means Heparin-Induced Thrombocytopenia with a relative platelet count fall (>50% of initial value) but it means also Heparin-Induced Thrombosis with life-threatening episodes in case of late diagnosis.

HIT diagnosis is determined by experts and well experienced physicians. It must be based on the combination of both clinical features and laboratory tests careful analysis. There is no gold standard criterion and a pre-test scoring system is proposed to evaluate HIT likelihood (4 T’s scoring system). Platelet count decrease kinetic is important and such analysis must be related to the context (medical or surgical) and the eventuality of clinical systemic acute reactions. This parameter is the main fishhook for HIT suspicion. In France, platelet monitoring performed twice a week is mandatory during all the period of heparin therapy. For HIT diagnosis, two type of tests are performed: functional tests (agregometry, ¹⁴C-serotonin release assay) able to detect antibodies activating platelets in presence of heparin and antigenic tests (ELISA enzyme-linked immunosorbent assay; rapid particle gel immunoassay) able to detect antibodies against PF4-heparin complexes. They are generally done by specialized laboratories. Both type of tests have their own limits and are complementary for HIT diagnosis.

HIT requires a multidisciplinary approach. The “4 S strategy” is warranted:
- If you Suspect HIT… you have to:
  - Stop heparin treatment…
  - Substitute by a non-heparin antithrombotic drug…
  - Survey the clinical evolution and treatment monitoring.

In conclusion, HIT diagnosis is difficult, time consuming and requires a technical expertise. After a nominative declaration to the local pharmacovigilance authorities, a certificate should be mandatory given to each HIT patient avoiding thus in the future any further deleterious heparin exposure.
SYM XVI.3: Management of patients with a history of HIT who require anticoagulant therapy

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HIT is an antibody-mediated, adverse effect of heparin that has a strong association with venous and arterial thrombosis. An increasing issue is the management of patients who present with a history of HIT and require anticoagulation. In general, in a patient with a history of HIT reexposure with heparin should be avoided. Especially if a patient has a history of HIT requires thrombosis prophylaxis, alternative anticoagulants such as danaparoid (750 U bid or tid sc), fondaparinux (2.5 mg od sc), or hirudin (15 mg bid sc) should be given as the risk of reoccurrence of HIT outweighs by far the risk of bleeding induced by alternative anticoagulants in prophylactic dose. The same is true for therapeutic dose anticoagulation in uncomplicated standard situations such as deep vein thrombosis (danaparoid, lepirudin, fondaparinux), coronary interventions (bivalirudin, lepirudin, argatroban), or chronic renal replacement therapy (argatroban, danaparoid, lepirudin). However, there are clinical situations in which alternative anticoagulants bear a high risk of adverse events. These are primarily cardiac surgery and major vascular surgery. In these patients all alternative anticoagulants have an enhanced risk for bleeding when given in therapeutic dose and for none an antidote exists. These patients should be treated depending on the time elapsed since acute HIT. HIT antibodies are transient, with the median time to antibody disappearance of 50 and 80 days. Great caution not to reexpose patients is therefore primarily needed within the first 100 days after acute HIT as in this time window reexposure can cause rapid-onset HIT due to still circulating HIT antibodies. However, when heparin is readministered in situations when HIT antibodies are no longer present, recurrence of HIT antibodies usually does not occur, and if HIT antibodies are regenerated, they occur no sooner than day 4-5. Since there is limited information regarding whether the overall risk of clinical HIT is greater in reexposed patients than in patients without a previous history of HIT, planned heparin reexposure should be restricted to the surgical procedure itself, and alternative anticoagulants should be used for preoperative or postoperative anticoagulation, if required. Several patients are reported who have undergone heparin rechallenge during cardiac or vascular surgery. None of the patients developed rapid-onset HIT. In patients who again formed anti-PF4/heparin antibodies this did not present a clinical problem as heparin was only used during surgery and not in the postoperative period. Despite the absence of large prospective studies the risk resulting from a potential boosting of HIT antibodies seems to be much lower than the risk of (peri)operative complications, especially major bleeding, associated with the non-heparin anticoagulants. While reexposure to heparin during cardiac surgery is an established regimen in patients testing negative in all HIT antibody assays, current studies address whether reexposure is also safe if only non-platelet-activating antibodies are present (i.e., EIA-positive but washed platelet activation assay-negative sera). For patients with acute HIT (thrombocytopenic, HIT antibody-positive) who require cardiac surgery, the following alternative anticoagulant approaches exist (in order of preference): delaying surgery until antibodies are negative; using bivalirudin; using lepirudin; using UFH plus the antiplatelet agent, epoprostenol; using UFH plus the antiplatelet agent, tirofiban; or using danaparoid.